The Technology and Chemistry of

ALKALOIDS

by

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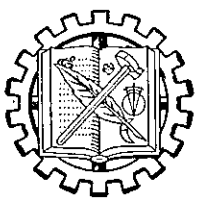
Consulting Chemist



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PREFACE

This book is primarily designed to help those engaged in or planning the manufacture of alkaloids. The main emphasis is upon the manufacturing processes rather than upon the chemistry of the alkaloids. Although the chemistry is dealt with as fully as possible within the framework of this book, of necessity many historical details regarding the development of the chemical structure of the alkaloids had to be omitted. Also, since this book deals mainly with the commercially practical alkaloids, it was felt that the purpose of the book would not be materially affected if the morphine and cinchona alkaloids were omitted.

The field of alkaloid chemistry and its technology, although rich in literature, is sadly lacking in many comprehensive works. This book was written to make available in one volume much practical information, such as the extraction and purification of the alkaloids, which heretofore had not been easily accessible. In addition, I wished to add what individual knowledge and experience I had acquired, hoping in this manner to be of some aid to those in the alkaloid industries and in the field of research.

I wish to express my sincere appreciation to Mr. Robert L. Brown for his guidance through the years and for his constructive criticism of this manuscript. I am also indebted to my wife for her invaluable help in proofreading and indexing.

F. E. H.

May 1950
New York, N. Y.

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CHAPTER I

INTRODUCTION

Alkaloids are physiologically potent compounds occurring in plants and containing nitrogen, usually as part of a cyclic structure. Ephedrine and colchicine are examples of alkaloids in which the nitrogen is of aliphatic character. They are usually found in seed-bearing plants (phanerogams), often among the dicotyledons and only rarely among the monocotyledons or the kryptogams (fungi, ergot). Different genera or families often form related alkaloids of a certain type or of several types which may not seem to be related at all. Alkaloids of a certain type of structure are sometimes found in different families of plants. For instance, the Solanaceae and Erythroxylaceae produce the tropane alkaloids, the nicotines, the hygrines, and the solanines. Nicotine-type alkaloids can also be traced to the Chenopodiaceae and the Leguminosae. Berberine or closely allied alkaloids are found in at least six different botanical families, the Ranunculaceae, Berberidaceae, Papaveraceae, Menispermaceae, Anonaceae, and Rutaceae. The formation of closely allied alkaloids within one species or among closely related species, or possibly even families, can be explained if one assumes that similar metabolic and enzymatic processes might be contributing factors. But then again it would be hard to explain the formation of similar alkaloids in different families of plants, unless their environment and their enzymatic and metabolic processes were similar. Partial support for this theory can be found in Hutchinson's diagram depicting the possible evolution of some plants. In that diagram the dicotyledons, the Ranunculaceae, Berberidaceae, Menispermaceae, Papaveraceae, and Anonaceae, but not the Rutaceae, are assumed to be closely related to each other.

Although some insight has been gained into the synthesis of alkaloids under physiological conditions, little or nothing is known of the part the alkaloids play in the metabolism of the plants. No rules can be made regarding the distribution of the alkaloids in the plants. They are usually found in almost all its parts, sometimes even in the seeds, and, although they are formed in living tissue, they are sometimes stored in dead tissue, such as the bark (cinchona) or the hull of the seeds. Nicotine is now known to be formed in the root of nicotiana, and after having

been transported to the shoots it is demethylated there to nor-nicotine. Partly because the alkaloids are formed where the metabolic activity of the plant is greatest and then seem to be cast aside, they have been considered to be detoxication products or by-products in the formation of proteins. On the other hand, they can also be thought of as being reserve materials for the formation of proteins, or to have some sort of hormone-like function or to regulate certain metabolic processes. They can also be thought of as protective substances. Resting seeds often contain alkaloids together with reserve protein, held in readiness for the onset of germination. However, in the germinating seeds of *Strychnos nux vomica* it has clearly been shown that their alkaloid content is dependent upon that very supply of protein. It is possible that alkaloids are formed during and as part of the metabolic breakdown of proteins. But other factors must certainly be assumed to be present and important, because a large number of plants, which seem to produce and consume proteins, appear to be void of alkaloids.

Robinson, in his important investigations concerning the biogenesis of alkaloids, stipulates the possibility of two important reactions which are possible under physiological conditions in the plants. One is the aldol condensation and the other is the condensation of aldehydes and ketones with ammonia or amines to form at first

$$=N—C(OH)=$$

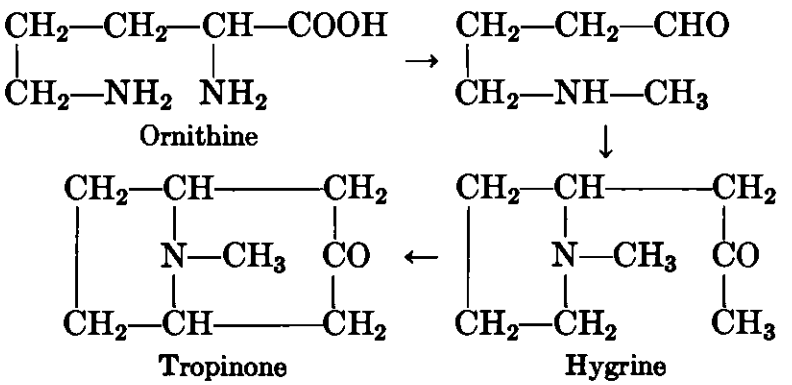
and further condensation with $=CH—CO—$ to give

$$=N—C—C—CO—$$

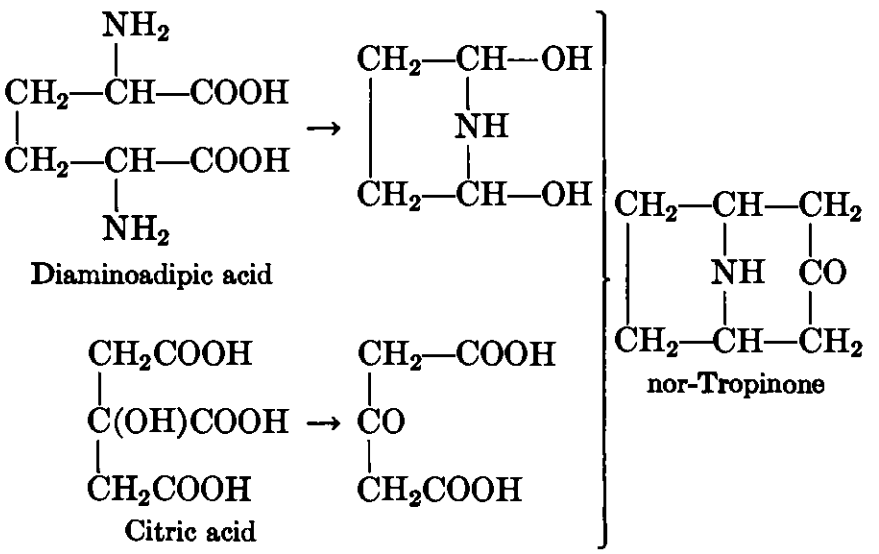
These reactions applied to a variety of amino acids as starting materials, and together with other reactions which are well known to occur under physiological conditions, have been shown to lead to a variety of alkaloids. Among the reactions taking part in the formation of alkaloids may be mentioned methylation and N-methylation (with the aid of formaldehyde and of methanol), oxidation (formaldehyde), decarboxylation, reduction, dehydrogenation, diene-syntheses, ring-enlargements, and possibly hydration and dehydration. Necessary for the performance of these reactions is the presence of amino acids, ammonia, formaldehyde, and products of the degradation of carbohydrates such as citric and acetone dicarboxylic acids. It is worth mentioning at this point that the alkaloids usually appear in the plant bound to some acid, which may even be characteristic to the plant (and the alkaloid), as in the case of aconitic (aconite), quinic (cinchona), or meconic (opium)

acids; other acids such as tannic, oxalic, succinic, or malic acids have been found more widely distributed.

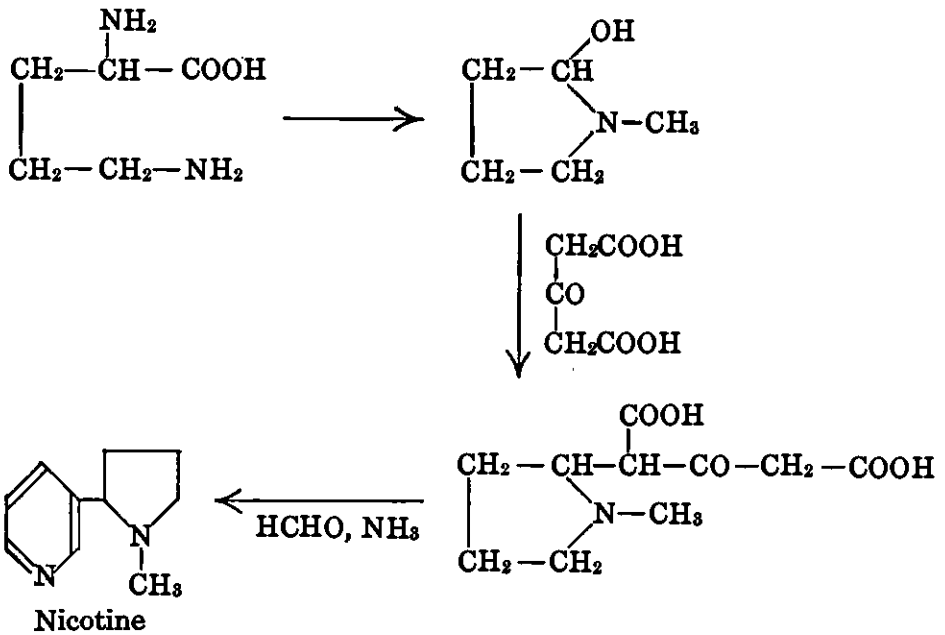
The apparently diverse and unrelated solanaceous alkaloids are thought to be derived from two simple amino acids, lysine and ornithine, with the latter obtained from arginine by hydrolysis. Hygrine could conceivably pass into tropinone through an intermediate product of oxidation.



nor-Tropinone and tropinone can be derived at from similarly simple products, from citric acid and from its oxidation product, acetone dicarboxylic acid.

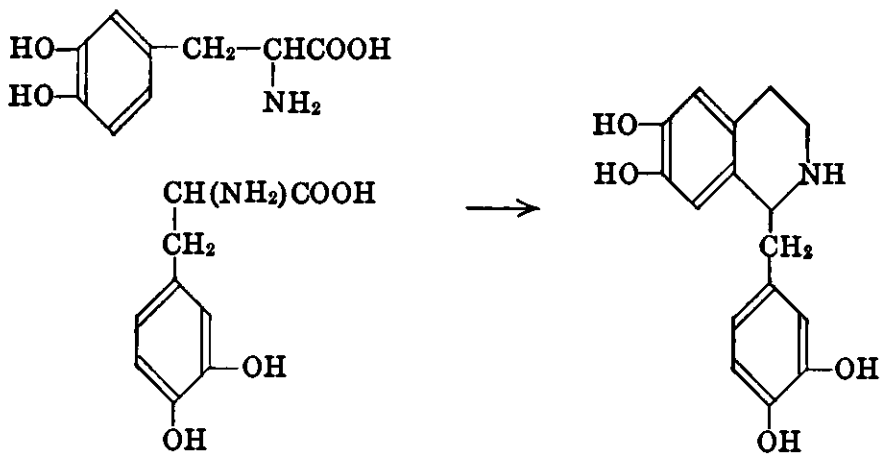


Nicotine, which also is found in solanaceous plants, can also be derived at from ornithine or from lysine or a proto-lysine derivative.

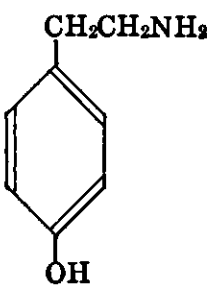


Nicotine

Isoquinoline alkaloids may be formed by condensation of two moles of di-hydroxy-phenylalanine. The initial product obtained is nor-laudanosine, but several other alkaloids can be derived from that base.

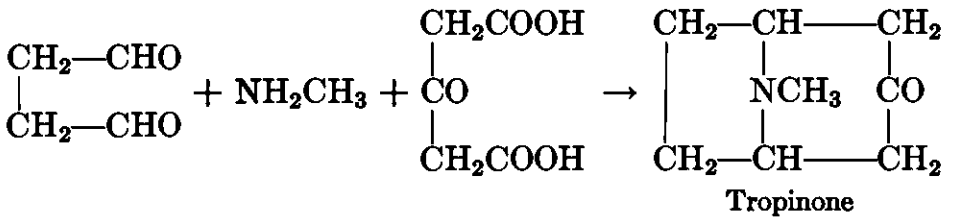


According to Barger the nitrogen in the heterocyclic ring and four of the carbon atoms of that ring are derived from an amino acid, probably tyrosine. Tetrahydro-isoquinoline derivatives can be derived at from tyramine.

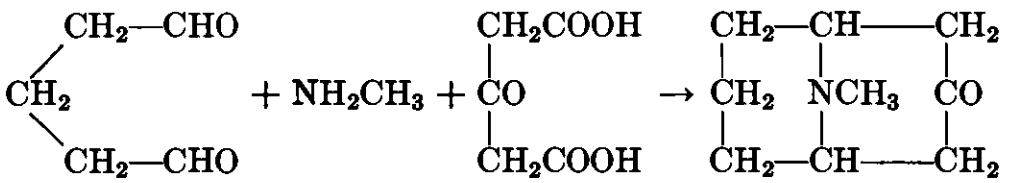


Tyramine

Alkaloid synthesis in vitro has been realized by Schoepf and his collaborators. Lobelanine, tropinone, and pseudopelletierine have been synthesized successfully with materials which probably exist in living cells and under approximately physiological conditions.



Tropinone



Pseudopelletierine

Alkaloids are, as a rule, white crystalline solids, containing carbon, hydrogen, nitrogen, and oxygen. Several alkaloids which do not contain oxygen are liquids. Other alkaloids such as berberine and sanguinarine are colored. Generally, the bases are practically insoluble in water but soluble in water-immiscible solvents. All alkaloids are basic owing to the presence of nitrogen in the primary, secondary, tertiary, or quaternary form, and the salts which can be formed are usually soluble in water and insoluble in water-immiscible solvents. Some alkaloids, such as colchicine, are very weakly basic and, in solution, are almost fully dissociated.

In addition to undergoing well-known and more or less precisely determined chemical changes and reactions, the alkaloids usually give empirically determined precipitation and color reactions.

Precipitations can be obtained with a variety of inorganic and organic reagents, sometimes from very dilute solutions. Attempts have been made to determine quantitatively some of the alkaloids by assuming a uniformity of precipitation under certain conditions, and also by assuming a uniform ratio in the precipitate between alkaloid and precipitant. Among the inorganic precipitation reagents may be mentioned potassium mercuric iodide (Mayer's reagent), bismuth potassium iodide (Dragendorff's reagent), chloroplatinic acid, auric chloride in hydrochloric acid, phosphomolybdic acid (Sonnenschein's reagent), mercuric chloride, iodine-potassium iodide, perchloric acid, Reinecke's acid (tetrarhodanato-diamino-chromic acid, usually as its ammonium salt), perchloric acid, and rarely potassium ferri- and ferrocyanide.

Some of the organic precipitants are picric acid, styphnic acid (trinitro-resorcinol), picrolonic acid (4-nitro-1-para-nitrophenyl-3-methyl-5-pyrazolone), naphthalene-beta-sulfonic acid, and sometimes tannic acid. Characteristic color reactions are obtained with the aid of dehydrating agents such as concentrated sulfuric acid; with oxidizing agents such as nitric acid; with a combination of these two or other agents which will dehydrate and oxidize simultaneously; and finally by treating with aldehydes or like compounds in the presence of dehydrating agents.

Definite relationships have been established correlating the constitution of some alkaloids with their physiological activity. In the solanaceous alkaloids, the mydriatic properties diminish and the anesthetic properties rise in the order atropine, benzoyl-tropine, and benzoyl-pseudotropine. Tropine and pseudotropine (as well as ephedrine) are mydriatics only in comparatively large doses. On the other hand, considerable changes can be undertaken in the tropine residue of the tropyl- and benzoyl-esters without impairing the activity of these compounds. The acid residue of the esters and its relative position to the nitrogen of the tropine residue seem to be of importance, and several N-hydroxy-alkyl-nor-tropanes have been synthesized to prove this point. Tropine esters of aliphatic acids were found to be void of mydriatic properties, which also were changed little when the benzene ring of the acid part of the ester was replaced by pyridine. The second ring of the tropine residue is thought to be of little importance, but scopolamine is not only a powerful mydriatic but also a central depressant. The importance of relative steric position of the tropine nitrogen and the ester group (or groups in cocaine) can be shown by the fact that derivatives of tropine and pseudotropine have distinctly different properties. Pseudo-atropine (tropyl-pseudotropine) is not mydriatic; the esters of pseudo-ecgonine are more potent local anesthetics and are less toxic. Patterns have been set up regarding natural and synthetic solanaceous alkaloids of opposite optical activity and their racemates.

Stedman has investigated the relation of the chemical constitution of physostigmine and similar compounds to their miotic activity. Methyl urethanes derived from basic nuclei substituted in the ortho position were found to be most active. Urethanes other than methyl and nuclear substitutions in other than ortho positions were found to be much less or totally incapable of producing miosis. Again it was found that optical configuration played an important part in the physiological activity of compounds.

Much progress has also been made correlating the structure of the cinchona and ipecacuanha alkaloids and of their derivatives and similar substances with their physiological activity.

CHAPTER II

PRODUCTION OF ALKALOIDS

Of the many methods which have been published, describing the manufacture of alkaloids from crude drugs, only a few are commercially feasible. The problems involved are found to be of a limited nature, provided the respective crude drugs used are at least of fair quality, proper equipment is available, and the directions are followed closely.

GENERAL METHODS OF EXTRACTION

Extraction with Water-immiscible Solvents

This procedure usually requires that the alkaloids, which are present in the plant in the form of salts, such as tannates or oxalates, be liberated by pretreatment of the ground drug with an alkali. Most common procedures use aqueous sodium carbonate, potassium carbonate, or ammonia, dilute solutions of which are used to moisten the drug thoroughly. Slaked lime has also been used at times.

a. The alkaloids are extracted with a solvent which is subsequently distilled. Solvents used are ethyl ether, isopropyl ether, and methylene chloride.

b. The alkaloids are extracted with a solvent from which they are subsequently removed by means of a dilute acid. Solvents used are ethylene dichloride, trichlorethylene, chloroform, and benzol.

The disadvantages of these methods are quite obvious. To effect intimate mixing of the drug with the alkaline solution, special equipment is needed, such as mixing machines with built-in spray-nozzles, tumbling barrels, or the like, although it is possible to perform this operation by hand. In either case expenses are incurred which cannot be overlooked in calculating the cost of manufacture. The cost of operating special mixing machinery, including man-hours necessary, will be found to be lower in the long run, as compared with mixing by hand, besides being more efficient.

Extraction with a water-immiscible solvent is tedious and not very effective, because deep penetration of the moist drug with the solvent

is unlikely. Action takes place mainly at the surface, and a large number of extractions are required, sometimes as many as ten to twelve, to remove all of the total alkaloids.

When ethyl ether or isopropyl ether are used, especially in heated extractors, the dangers of fire and explosion are always present. Use of chlorinated solvents considerably lessens these dangers, but a very considerable quantity of solvent will be lost as residue in the exhausted drug. This loss can amount to as much as 25–30% of the initial crude drug used. Furthermore, chlorinated solvents, and especially benzol, constitute a considerable health hazard and can only be used if sufficient ventilation is provided.

Extraction with Water-miscible Solvents

The ground drug is extracted with a solvent, such as methanol, ethanol, or isopropanol, which is subsequently distilled.

No pretreatment of the drug with alkali is required, since alcohol seems to dissolve equally well the alkaloidal salts and the free bases present in the plant. It is believed that alcohol exhibits a hydrolyzing effect upon the alkaloidal tannates and other salts. Some investigators have recommended pretreatment of the drug with alkali; others have recommended acidification of the alcohol with a small amount of mineral acid or an organic acid; but neither was found to be necessary.

Penetration of the drug with alcohol is complete, and a maximum of four extractions is necessary to remove all of the alkaloids. Somewhat less solvent is lost with the finally exhausted drug than when chlorinated solvents are used. Health hazard and fire danger are small.

This method of extracting the total alkaloids seems to be most efficient and can be highly recommended.

Extraction with Water

The drug is extracted with acidified water, which is then made alkaline, and the alkaloids are removed with an immiscible solvent. Dilute solutions of hydrochloric acid, sulfuric acid, and acetic acid are used.

Although this is undoubtedly an excellent and inexpensive solvent for alkaloids, it possesses the disadvantage that it also dissolves large quantities of other materials present in the plant, such as sugars, starches, coloring matter, proteins, tannins, and resins, which serve as annoying impurities in the later stages of purification.

The possibility of removing the alkaloids from the aqueous extract by the use of selective adsorbents is highly promising and has been used with some success in the case of quinine.

GENERAL METHODS OF PURIFICATION

Crude extracts are obtained when the ground drug is extracted with a solvent, and the extract is concentrated. The term "crude extract" is usually applied to the syrup so obtained by extraction with alcohol.

The crude extracts contain the "total alkaloids," resins, fats, coloring matter, tannates and salts of organic acids such as oxalic acid, and other

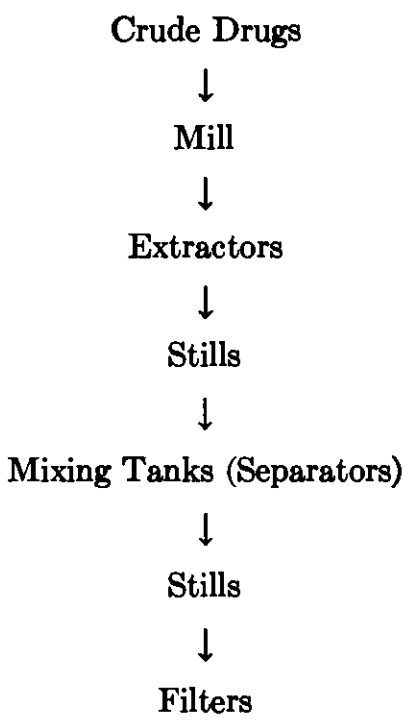


FIG. 2.1. Type of equipment used (diagrammatic).

impurities. The amount of these by-products varies not only in different plant species but also with different parts of the plant. Seeds, for instance, contain the highest amount of fatty matter of any part of the plant, while extracts from the root, as a rule, contain the least amount of impurities.

Preliminary purification of the alkaloids, which are in a highly impure state in the crude extract, can be effected by the use of different immiscible solvents. Use is made of the general principle, which holds true for most alkaloids, that their salts with mineral acids are insoluble in most water-immiscible solvents but are soluble in water, whereas the free bases usually are soluble in water-immiscible solvents but are insoluble in water.

A certain amount of purification can thus be accomplished by mixing the crude extract with a dilute mineral acid and treating the resulting

mixture of dilute acid, containing the alkaloids, and of the fatty matter and other impurities, with a solvent such as ethylene dichloride, trichlorethylene, or methylene chloride. The solvent will dissolve most of the fats and some other impurities, while the alkaloids remain in the acid aqueous phase. Tannates and tannic acid, which will separate from water at a pH slightly below 7, are insoluble in most solvents and must be filtered off. If the aqueous phase is now made alkaline, the alkaloids can be removed by extraction with a solvent, such as chloroform or ether, leaving behind in the aqueous phase most of the coloring matter.

Preliminary purification of the alkaloids is always undertaken on a large scale. Emphasis will be placed on plant or large-scale equipment, which is specialized, and construction and installation of which are not very well known. Final purification consists of one or more recrystallizations and is usually undertaken on a pilot-plant scale or in the laboratory. The standard equipment necessary for these operations need not be described in great detail.

Both adsorption and ion-exchange have been applied successfully to the manufacture of alkaloids. They may be applied whenever small amounts of dissolved material are to be removed from a large amount of solvent. Activated charcoal has been used to remove alkaloids from aqueous solutions. Elution of the latter can be effected either by means of a different solvent or with an aqueous solution of a salt at a different pH. Although elution with a solvent is to be preferred, it has been found to be rather difficult in both instances. A mixture of kaolin and asbestos has been used to adsorb cinchona alkaloids.[1]

Ion-exchange processes have been investigated in reference to scopolamine, morphine, and cinchona alkaloids, and considerable success has been reported.[2] Sulfonated coals and resins are the materials usually used. The alkaloids are removed from their aqueous solutions, and they can be eluted either with dilute mineral acids or with a solvent which has been preceded by a suitable alkaline liquid. Hydrated magnesium silicates have also been used.[3] Other more recent patents in connection with ion-exchange processes have been issued.[4]

The crude drugs of commerce are handled in bulk, either loose or pressed into bales. In either instance the drug must be powdered to facilitate extraction. Provision must then be made to extract the ground drug, and to process and purify the extracts.

Equipment essential in the manufacture of alkaloids consists therefore of devices for grinding, devices for extraction, separators for extractions of liquids with immiscible solvents, and stills for distillation of solvents.

EQUIPMENT AND MACHINERY

The equipment described on the following pages is basic equipment, necessary for the manufacture of most alkaloids.

The Mill

Almost any mill of the hammermill type will be found suitable. Feedmills have been found to be highly efficient and durable, besides being relatively inexpensive and economical in operation. Screen

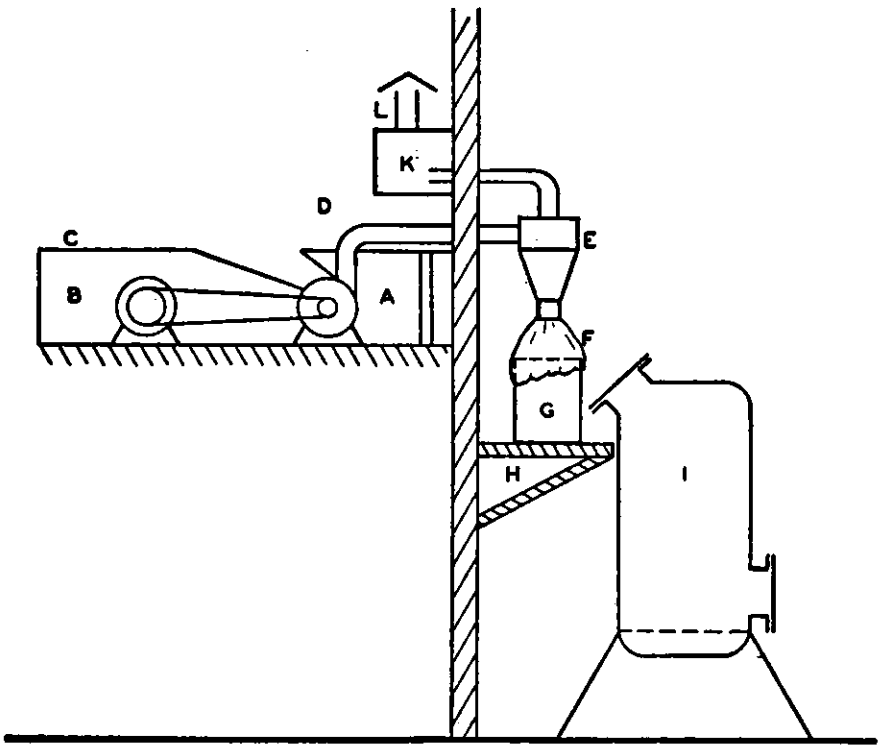


FIG. 2.2.

A. Mill
B. Motor, explosion proof
C. Working bench
D. Blow line
E. Dust separator
F. Canvas sleeve
G. Open-top 55-gal. drum
H. Loading platform leading to all extractors
I. Extractors
K. Wooden box
L. Air outlet

openings of ¼–5/16 in. will yield the right coarseness of grind at about 1200–1500 rpm, or whatever the maker of the mill specifies. The motor needed will have to be of at least 3 hp and must be explosion proof. The mill is best installed in a separate room, on the floor (Fig. 2.2). The bales or bags are lifted on a strong wooden workbench, about 3 ft high, where the material is broken up by hand and searched for rocks and

other foreign material that might break or jam the mill. The drug is pushed into the feeder as fast as the mill can handle the material. Experience has shown that this type of mill can handle even the hardest drugs, such as yohimbe bark. The knives and the screen will wear out in time but can usually be easily replaced.

The milled drug is blown automatically into the next room where the extractors are, and where the material can be collected on an elevated platform. This blow line, which should be at least 4–5 in. in diameter, is best made of ordinary galvanized sheetiron of suitable strength. Joints should be sealed with 2-in. tape. The dust separator, which is in the next room also, should have the air outlet leading to the outside of the building into a wooden box. Some additional material can usually be collected there.

The operator, while grinding, must make sure from time to time that the line is free and unobstructed. This can usually be judged from the sound of the mill when in operation. A lower pitch usually means an obstructed line. If that should happen, banging the blow line with a wooden stick, while the mill is running, will usually free the blow line.

The ground material is collected in open-top, 55-gal drums which are connected to the dust separator by means of a canvas sleeve, firmly secured with a rope. From the drums the ground drug is dumped into the extractors.

The Extractors

The extractors are iron tanks the capacity of which can be varied from 400–4000 lb or more to suit individual needs. The height of the working space should exceed slightly its width. The working capacity for a charge of about 450 lb, being about 560 gal, would then be about 4 ft in diameter and 6 ft in height (Fig. 2.3).

The false bottom is created by means of a supported, iron, perforated plate, ⅛–¼ in. thick. Perforations are ¼ in. in diameter and ½ in. apart (center to center). This is covered with a stainless steel wire screen, 60–80 mesh fine. The extractor is equipped with a bottom outlet, a jacket for heating with steam (5–10 lb of steam pressure), one to several inlets near the top for charging the solvent, and an attachment at the top for applying compressed air, including a separate air release. Two manholes are provided, as shown in the diagram, to facilitate charging of the extractor with the drug and removing of the exhausted marc. To assure easy access, the manholes should be at least 2½ ft wide. For continuous countercurrent operation, as outlined elsewhere, four extractors are needed.

Basket-type extractors have also been used successfully. They are

similar in construction and operation to the conventional extractors, but the installment and maintenance of additional machinery to lift and move the baskets must be considered. Side and steam-jacket of the percolator are identical with the one in Fig. 2.3, but the lower manhole as well as the upper one is eliminated. Instead the whole head is

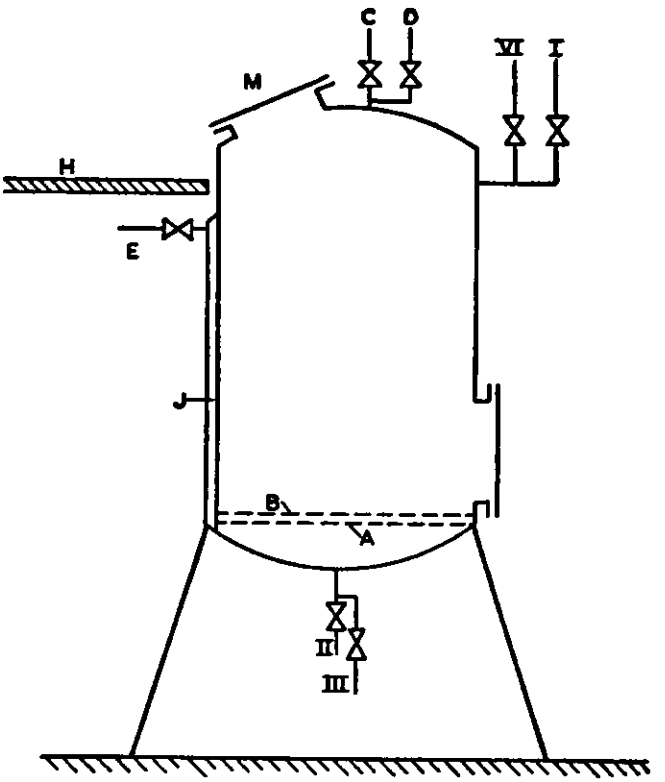


FIG. 2.3.

I. Connection from line I (Fig. 2.6)
II. Connection to pipeline II (Fig. 2.6)
III. Connection to pipeline III (Fig. 2.6)
VI. Connection from line VI (Fig. 2.6)
A. Perforated plate
B. Stainless steel screen
C. Compressed air (Line VIII Fig. 2.6)
D. Air release (Line IX, Fig. 2.6)
E. Steam connection
J. Jacket
M. Manholes

removable. An asbestos gasket should be used to ensure a tight connection at that point. The perforated plate and screen are replaced by one or two simple bars on which the basket rests. The basket is cylindrical, and the sides as well as the bottom are made of rolled, perforated iron or steel, $\frac{1}{8}$–$\frac{3}{8}$ in. thick, $\frac{1}{4}$-in. perforations about $\frac{3}{4}$ in. apart, somewhat less at the bottom. The inside is lined with 60-mesh stainless steel screening. Provisions must be made at the top for fastening of hooks to facilitate lifting of the basket. The basket is charged as usual. It

is emptied by lifting it from the extractor and simply dumping its content into a suitable receptacle. Because drainage of the marc is achieved by gravity only, the loss of solvent is somewhat higher in this type of extractor as compared with the type previously mentioned.

Considerable progress has been made in recent years in the designing of rotary, centrifugal, and continuous countercurrent types of extractors, and a survey of patents covering these items and related machinery and some processes is given.[5]

Pumps

One or two centrifugal pumps are needed in the continuous system of extraction. They are usually made of iron and have a ¾-in. feeding line. They are used for charging extractors and for circulating extracts. Their motors must be explosion proof. Their function is explained in the diagram (Fig. 2.4), where it will be noticed that installation of the second pump is optional, but desirable where extractors with a working capacity of 1000 lb or more are used.

Stills for Concentrating Extracts

Two stills are desirable to assure efficiency in the continuous process. They differ in their respective capacities only, which vary in relation to the size of extractors used. Calculated for extractors of 450-lb capacity, the stills should be of 500–600 gal and of 150–200 gal capacity respectively. They should both be jacketed, because the use of coils for heating has been found to be of great disadvantage and cannot be recommended. Material tends to cake on the coils and will eventually char, with the effect that heat transfer will become less effective and valuable material will be lost. The stills should be glass-lined or made of stainless steel. Iron stills cannot be recommended. Copper stills have been used, but they do not offer enough of an advantage to warrant their high cost of installation.

The stills should be equipped with a stirrer, rotating at 30–50 rpm, and having one small propeller with three or four blades. The condensers should be of the coil type, and as efficient as possible. If two stills are being used as recommended, the larger still does not need a vacuum receiver but nevertheless must be equipped with vacuum to facilitate charging of the still, and with a line for compressed air to facilitate transfer of the concentrate to the smaller still for final concentration under vacuum. The smaller still can also be used for recovery of solvents, such as ethylene dichloride or chloroform. Arrangements must then be made in the piping of the bottom outlet for discarding those residues. When the still is to be charged with a solvent for recov-

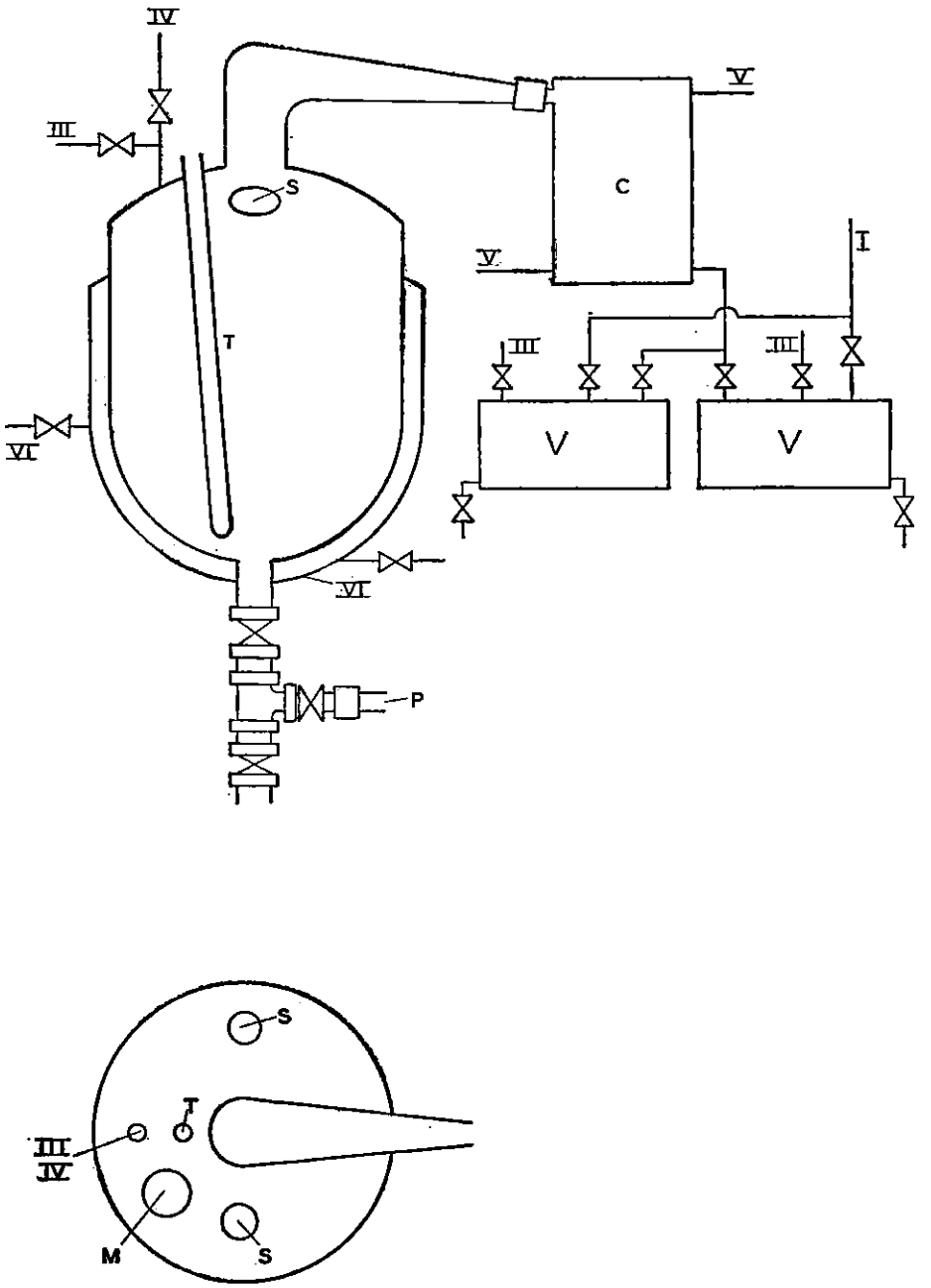


FIG. 2.4.

C. Condenser
M. Manhole
P. 1½″ connection to pressure filter
S. Sightglasses
T. Thermometer well
V Vacuum receiver

I. Vacuum line
II. Compressed air line
III. Air release
IV. ¾″ connection for charging under vacuum
V. Water
VI. Steam

ery, this is done by means of vacuum through a ¾-in. rubber hose attached at the top.

Mixing Tanks (Separators)

A minimum of two tanks is necessary. They were found to be most useful when constructed with a pitched or round bottom to facilitate separation of immiscible solvents. It was found to be most advantageous when they were made of stainless steel or were glass-lined. They must be equipped with an efficient stirrer, which should be of stainless steel also. Gate valves of ¾ in. are used at the bottom outlets. The size of these tanks again depends on the amount of drug which is to be handled. When four extractors with a capacity of 450 lb are used continuously, mixing tanks of 300–400 gal will be adequate.

Filters

Filters needed in the process of manufacturing alkaloids are usually made of stoneware and should be available in different sizes from 24 to 48 in. in diameter. It was found best to have two or more filters available of each size. Heavy canvas, which has been boiled with water several times prior to cutting it to the right size to remove starch and sizing, can be recommended instead of filter paper or asbestos.

Stoneware Crocks

A variety of crocks of different sizes, from 10–100 gal capacity, are necessary, some of which should be equipped with bottom outlets.

Stills for Processing Semi-finished Alkaloids

Two small stills, one of 50-gal and one of 25-gal capacity, play a most important part in the final stages of the manufacture of pure alkaloids and their salts, and they should be of the very best quality. The following features have been found necessary for smooth and efficient operation: The stills should be constructed of either stainless steel or they should be glass-lined. They should be jacketed and equipped with a 2-in. bottom outlet with gate valve. They should have two sight-glasses, one for illumination and the other for observing the material in the still. They should have an efficient coil condenser and one or two vacuum receivers with connections for vacuum and compressed air. They should be sufficiently elevated from the floor to allow attachment of the pressure filter, and to allow placing of a 2–5 gal container underneath the still for emptying purposes. They should be equipped with a thermometer well and with an attachment for charging under

vacuum. Details of the construction can be seen in the diagram (Fig. 2.4).

Pressure Filter

The pressure filter has been found most efficient whenever alkaloidal solutions have to be treated with charcoal because (1) they are time

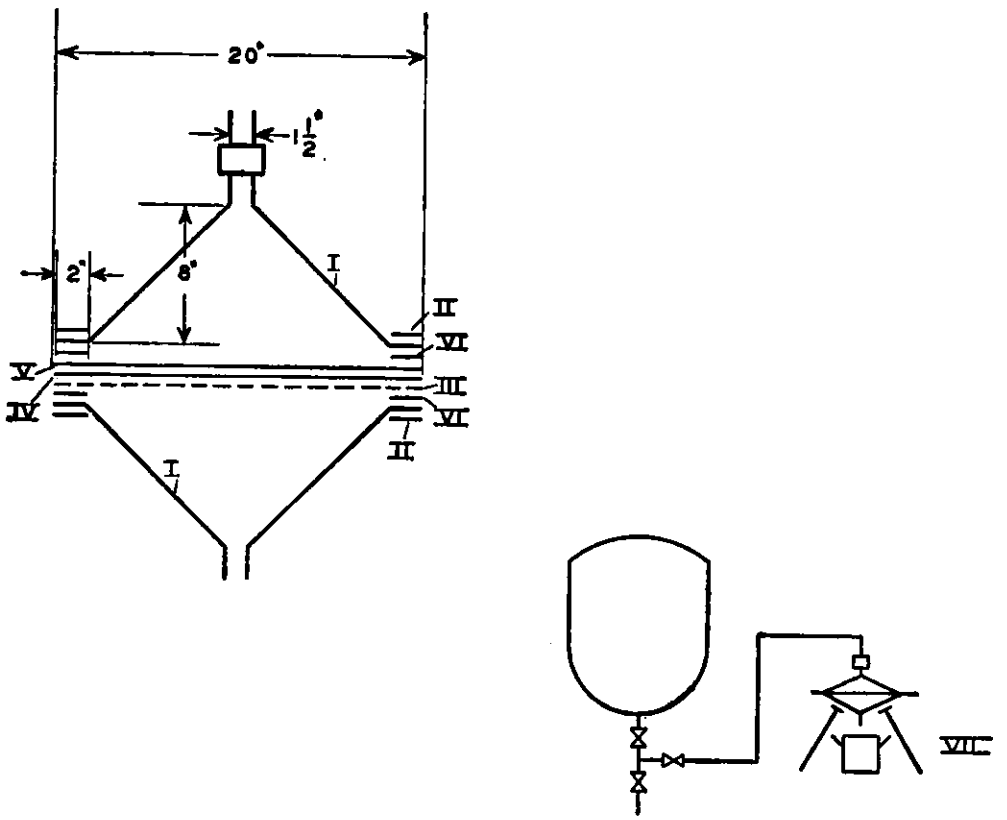


FIG. 2.5.

I. Stainless steel cones, flanged
II. Iron reinforcement rings
III. Perforated plate
IV. Heavy cloth
V. Three sheets of filter paper
VI. Rubber rings
VII. Support
VIII. C-clamps (not shown)

saving, (2) they are absolutely clean, and (3) complete washing of the charcoal cake can be accomplished with a minimum of liquid. Either hot or cold solutions can be filtered, and when filtering hot solutions the loss of solvent by evaporation is kept to the minimum.

The filter, construction of which can be seen from the diagram (Fig. 2.5), is made of $\frac{3}{16}$-in. stainless steel. The perforated plate in the center should have $\frac{3}{16}$-in. holes drilled $\frac{1}{2}$ in. apart, or $\frac{1}{8}$-in. holes $\frac{3}{8}$ in. apart. The outer edges are reinforced with iron rings, $\frac{3}{16}$ in. thick, which serve to equalize the pressure of the C-clamps, eight to ten of

which are needed to hold the filter tight. Leakage is prevented by the use of rubber washers in the places indicated. A support for the filter must be constructed as needed.

Two pressure filters of different sizes are indicated, one of 15-in. diameter attached to the smaller still, and one of 25-in. diameter attached to the larger still.

To prepare the filter for filtration, the perforated plate is first covered with one layer of heavy canvas, then with three layers of filter paper. It is secured tightly and attached to the still as shown in the diagram. A pressure of 10 lb in the still is sufficient for filtration. Charcoal alone is never used, but an equal amount of a filter aid is added to assure clean and fast filtration. The first 1–2 gal of filtrate often contain some charcoal and are returned to the still for refiltration. When filtering, pressure is first brought up in the still before opening the valve to the pressure filter. It is economical to blow the charcoal cake almost dry with compressed air before washing it.

Vacuum Pump

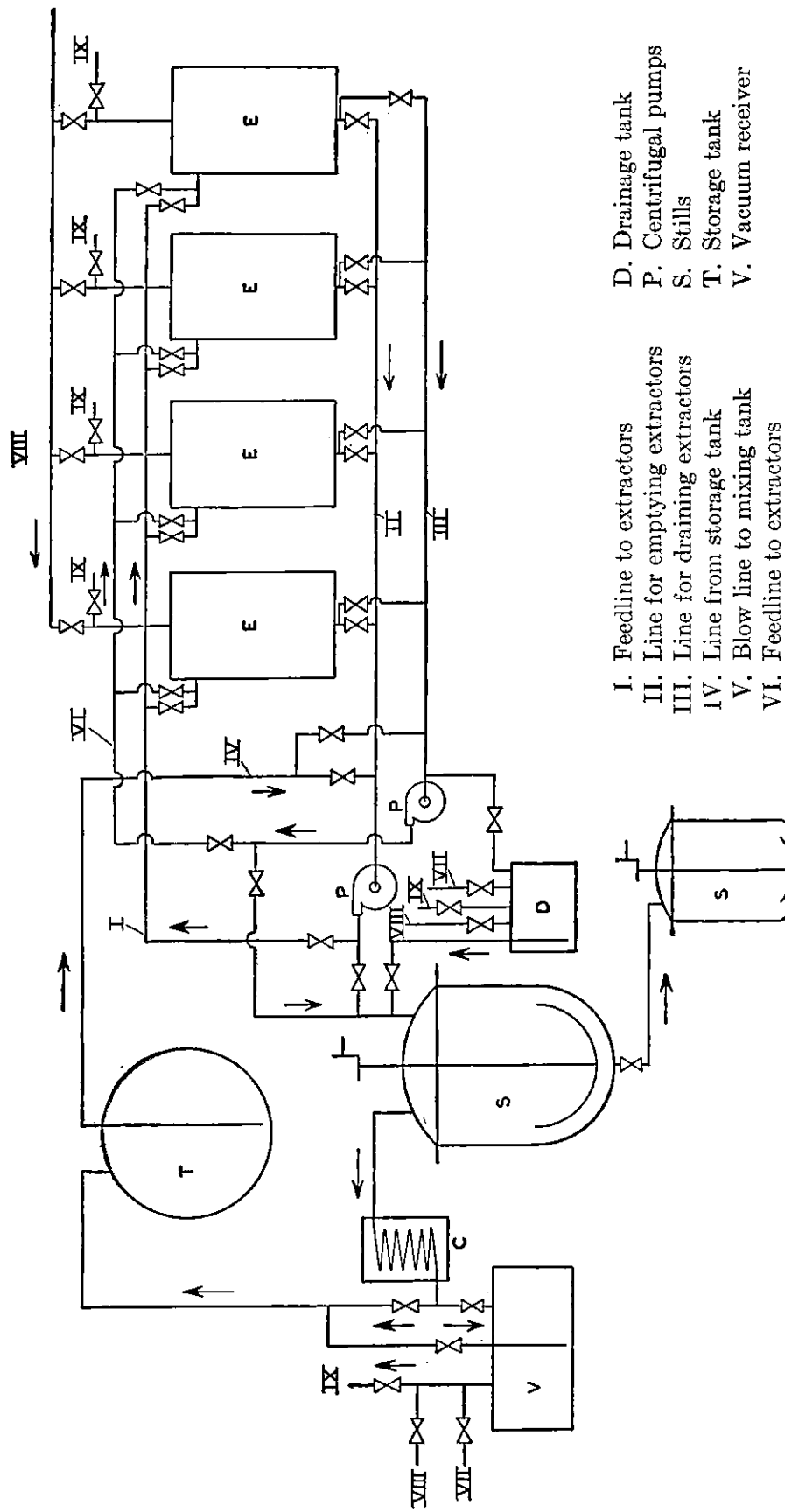
Any water- or steam-operated vacuum pump can be used, including water-sealed mechanical pumps, provided the volume of evacuation is sufficiently large. Oil-immersed pumps cannot be recommended, because solvent vapors will soon be adsorbed by the oil and render the pump inefficient. Separate pumps and separate vacuum lines for filtration and for vacuum distillation are indicated.

Other Equipment

The following equipment is also needed: an air compressor, having a fairly good capacity and delivering at least 25 lb of pressure; one or more containers, 10–25 gal capacity, made of stainless steel or glass-lined, preferably with cover; one large refrigerator; a steam drier, consisting of a wooden cabinet, with shelves, heated by means of a steam coil in the bottom (15 lb of steam), with an appropriate outlet for the moist air, the shelves consisting of wire screen nailed onto a suitable wooden frame.

General handling equipment includes pails (2½–3 gal) and dippers (2 qt), either enameled or of stainless steel. Enameled or glass trays should be used for drying.

For transfer of larger amounts of liquor it was found advisable to have a small centrifugal pump (½-in. lines) mounted on a small platform on casters. The explosion-proof motor is equipped with a 25-ft extension wire, and the pump is equipped with two 25-ft sections of garden hose.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D. Drainage tank
P. Centrifugal pumps
S. Stills
T. Storage tank
V. Vacuum receiver
I. Feedline to extractors
II. Line for emptying extractors
III. Line for draining extractors
IV. Line from storage tank
V. Blow line to mixing tank
VI. Feedline to extractors
VII. Vacuum line
VIII. Compressed air line
IX. Air release

FIG. 2.6.

PLANT LAYOUT

Plant layout for the production of alkaloids varies little from that of most chemical plants, except greater emphasis is placed upon two precautionary measures:

1. The grinding of the crude drug must be accomplished sufficiently far away from the finished product as to avoid any possible contamination.
2. Adequate ventilation must be provided, since the volatile solvents used are a possible health and fire hazard.

Only the installation and hookup of the extractors have to be considered here in any detail (Fig. 2.6). The piping has been designed in such a way as to make possible transfer of liquids from storage tank to extractors, from extractors to still (including provision for sufficient drainage), from one extractor to another, and for circulation of liquids.

PLANT OPERATION

Operation of an alkaloid-producing plant does not differ appreciably from that of any other chemical plant, except for the initial stages of extraction. Much of the success in producing alkaloids depends on how efficient and economical that operation can be performed.

All crude drugs should be stored in a dry place prior to milling and, if possible, should not be stored too long. It was found that the yield of alkaloids depends to a great extent upon the condition of the crude drug used. If the material is stored in a damp place, it will soon deteriorate by rotting because of moisture, but, even if it is stored in a dry place for a long time, invisible deterioration due to old age will set in. In either case it becomes increasingly difficult to isolate the amount of alkaloid as represented in the figure for total alkaloids as determined according to specifications, not only because of actual decomposition of alkaloids, but also because of the large increase in impurities which render isolation and purification of the alkaloids difficult.

Wherever possible, drugs should be milled just prior to extraction and should be charged into the extractors soon thereafter. A mesh size of 20–30, or possibly down to 40, is most advantageous. If the powder is too coarse, complete extraction of the alkaloids is very difficult. If the powder is too fine, the material will pack together excessively and "channeling" will prevent the solvent from penetrating to all parts of the extractor. Therefore, extraction will be incomplete again.

The powdered drug is charged into the extractors and is immediately ready for extraction if alcohol is to be the extracting medium. Cold

alcohol is pumped in from the storage tank, at a moderate rate, until the batch is completely covered with about 4 in. of supernatant liquor.

For an initial extraction, that is, an extraction of ground drug which has not previously been moistened with alcohol, an average of ¾ gal of alcohol will be needed per pound of drug. The amount varies with the nature of the material, the coarseness of the grind, and the amount of dead space in the bottom of the extractor. For further extractions, less alcohol will be used, depending on the nature of the drug and how well it is drained of the previous extraction.

Circulation of the extracting medium is desirable but not absolutely necessary. It will not reduce the number of extractions necessary, but it will assure a complete and thorough extraction of the total alkaloids present. To effect circulation, the extract is drawn off at the bottom by means of the circulating pump, and is returned at the top of the extractor through the feeding line. While circulation is in progress, heat can be applied through the jacket until all of the batch, liquid and solid, has reached a temperature of about 50° C. The heat can be shut off once the whole extractor has been thoroughly warmed, although it was found advisable to continue circulation for another hour at least. For reasons of safety, health of operating personnel, and economy, boiling of the alcohol should be avoided. The yield of total alkaloids will not be affected if the extracting medium is only warmed but not boiled.

Upon completion of circulation, the material should be allowed to macerate overnight. Covering the upper manhole of the extractor is advisable, to keep the loss of alcohol by evaporation to a minimum.

For reasons of economy and simplicity of operation, a continuous system of countercurrent extraction has been found to be most useful. Its advantages are quite clear. A minimum number of extractors and extractions are necessary, and a minimum amount of alcohol has to be distilled or, in other words, the best possible use is made of each given amount of alcohol. On the other hand, each extractor is used fully all the time.

Systems using four, six, and eight extractors have been devised and described, but only a four-extractor system will be described here. In the diagram (Fig. 2.7) the extractors have been identified by letters, A through D. The arrows to the left denote extracts which are being drawn into the still for concentration. It will be noted that only first and second extracts are being distilled, third and fourth extracts are being used further. When the system is in its fourth day of operation or later, fresh alcohol is being used only for first and fourth extractions. The numbers near the arrows denote the number of extraction, either

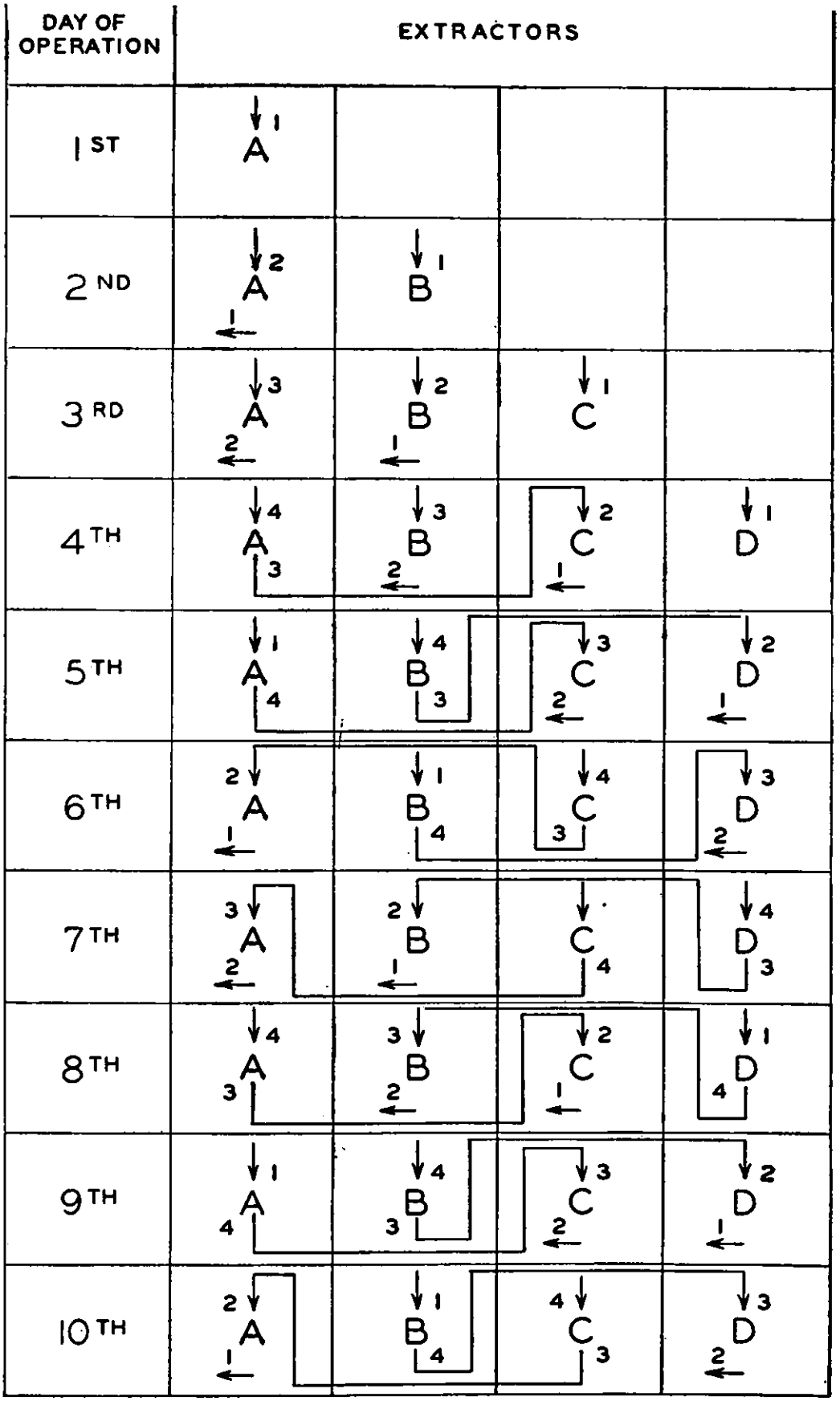

FIG. 2.7. Flowsheet of continuous countercurrent extraction.

being drawn from or being charged into the extractor, with reference to the batch in that extractor. Thus, for instance, on the third day of operation, the second extraction from "A" and the first extraction from "B" are being transferred to the still for concentration. On the fourth day, the second extraction from "B" and the first extraction from "C" are transferred to the still, the third extraction is drawn off "A" and charged into "C," whence it becomes a second extraction. On the fifth day of operation the fourth extract off "A" is charged into "C," whence it becomes a third extraction, and so on. The following daily schedule of operations is based on the system just described and has been found very efficient:

First thing in the morning, those extractions which are ready for concentration (firsts and seconds) are being transferred to the still, and distillation is started. Next in importance is the emptying of that extractor which is to be recharged that day. The third and fourth extractions are drawn off into the appropriate extractors, which have been made ready previously, and the exhausted marc is subjected to compressed air to recover as much alcohol as possible. After tightly securing the top manhole cover, compressed air is put on for as long as time permits. Next, circulating and heating are begun with those batches, or extractors, which are ready for that type of operation. Finally the exhausted marc is discarded, the right amount of drug is ground, charged into the extractor, covered with fresh alcohol, heated and the liquid circulated. With reference to the first and second extractions, which are being drawn off into the still, it might be worth while to mention that approximately 2–3 hr should be allowed to drain most of the liquid.

Effective, and therefore economical, operation of the continuous system depends almost entirely upon the efficiency of the still. Considering as an example a system of four extractors of 500-lb capacity each, a still of 500–600 gal capacity is the minimum, the condenser, in this case, having a capacity of about 350 gal per working day. This can be either the straight working day or a 24-hr continuous operation, during which time about 300–360 gal should be distilled. If the still or the condenser cannot handle that amount, which, of course, is correspondingly larger if larger extractors are used, the system loses its continuity and becomes, therefore, ineffective and wasteful.

Concentration of the extracts is best undertaken at normal pressure, with a steam pressure of about 10–15 lb applied at the jacket, with continuous stirring. The recovered alcohol is returned to a storage tank of adequate capacity. Distillation can be effected, of course, under vacuum. In that case a vacuum of about 15–20 in. will be found highly effective, as it will increase the rate of distillation more than

threefold. At the same time, however, the loss of alcohol will mount considerably and may be as high as 20–30% of the amount charged into the still. As a rule, distillation under vacuum has been found uneconomical, despite the fact that smaller equipment can be used and that the operating time is cut considerably.

When most of the alcohol has been removed in the course of a day, the rate of distillation will drop. At this point the still is shut down, and it is now ready to receive the next charge. It is of advantage to check the temperature of the batch in the still. For most alkaloids, the concentrate should not be heated above 80–85° C.

In this manner, 5-day charges, or extracts, are being accumulated in the still. At the end of that time, when most of the fifth day's charge has been evaporated, a residue of about 70–150 gal per 1000 lb of extracted drug should be present, the amounts varying considerably.

To facilitate the isolation of the alkaloids from that residue, the rest of the alcohol should be removed under vacuum, with continuous stirring, until the rate of distillation has dropped to a mere trifle. When applying vacuum, care must be taken that the charge in the still is not too hot, otherwise it might boil over.

This operation of final concentration will take from one to several hours, depending upon the size of the individual batches and the efficiency of the equipment used. During that time the still cannot be used for distillation of that day's extract, and time will unavoidably be lost there. For that reason it is desirable to use two different stills for evaporation and final concentration. The extracts are being concentrated and accumulated in the larger still, and when ready for final concentration under vacuum the material is transferred to a smaller still where that operation can be undertaken, thus freeing the larger still for continued distillation of fresh extracts. If no still is available for the final concentration, the system of continuous extraction is being held up for one day while vacuum concentration is being undertaken.

When most of the alcohol has thus been removed under vacuum, the still is allowed to cool for ½–1 hr, and approximately equal volume of carbon tetrachloride is added to its content. Stirring is continued for ½ hr, and the diluted residue can now be transferred to a mixing tank. The purpose of the solvent is not only to serve as diluent of the extract, but to facilitate easier removal of the total alkaloids, as well as to keep fats and resins in solution.

The operation of transferring the carbon tetrachloride solution could be done manually, but for reasons of health of the operating personnel it is best to blow the batch by means of compressed air. A line of 1½–4 in. in diameter should be set up permanently for this operation, leading

from the bottom of the still to the top of the first mixing tank. Recommended construction is described in the diagram. An air pressure of 15–20 lb will be sufficient. The still should be equipped with an outlet for release of pressure with a pipe leading to the outside. Stirring is continued during the process of blowing over.

In the diagram (Fig. 2.8) the valves have been numbered A, B, and C, and the following procedure of operation is recommended: (1) Keep A closed, open C, and blow steam through blow line, making sure that it is clear. (2) Close C, open A, and blow steam into the still under continuous stirring, making sure that the outlet is clear. (3) Close B, keep A open, open C, and apply compressed air to the still.

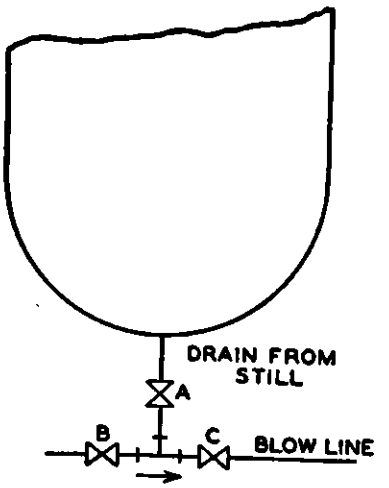

FIG. 2.8.

If the extract, after dilution with carbon tetrachloride, is extremely lumpy and defies all attempts to blowing over, about ¼ of the total volume of warm water may be added. Heat lightly, stir for ½ hr, and then proceed to blow.

The solution of extract in carbon tetrachloride is ready for further purification and isolation of the total alkaloids. The method just described for obtaining crude extracts can be used for the manufacture of most alkaloids; slight variations will be discussed in the different chapters.

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2. Applezweig, *J. A. C. S.* **66**, 1990 (1944). Applezweig and Ronzone, *Ind. Eng. Chem.* **38**, 576 (1946).
3. U. S. Patent 1,300,747.
4. U. S. Patents 1,447,400; 2,009,181; 2,067,324; 2,072,089; 2,132,945; 2,293,954.
5. U. S. Patents 2,405,158; 2,400,951; 2,377,135; 2,347,322; 2,218,993. British Patents 584,232; 569,499; 569,498; 565,405; 563,122; 547,772; 546,675; 512,660. German Patents 736,281; 725,482; 715,248.

CHAPTER III

THE ALKALOIDS OF ACONITE

CHEMISTRY

Numerous alkaloids have been found in the species of aconite, some of them isomeric or possibly identical, but relatively little is known of their chemical constitution to this date. On the whole they can be classified into two groups: (a) The true aconitines, which are extremely toxic esters of amino-alcohols, and (b) the atisines, which are of lower molecular weight and are less toxic.

The True Aconitines

These alkaloids can be distinguished by their relatively large molecular weight. They have a single nitrogen atom, usually four methoxyl groups, and two additional hydroxyl groups which are esterified with acetic acid and another aromatic acid, such as benzoic, anisic, veratric, or anthranilic acid. The amino-alcohol aconine, obtained upon hydrolysis, is thought to be common to all aconitines.[1]

Aconitine. This alkaloid, $C_{34}H_{47}O_{11}N$, was first obtained by Groves [2] and later by Wright *et al.*,[3] Dunstan *et al.*,[4] and Freund *et al.*[5] Majima *et al.*[6] considered it to be identical with japaconitine, isolated by them. This view was shared by Mandelin,[7] Luebbe,[8] and Freund and Beck,[9] but was opposed by Paul and Kingzett,[10] Wright *et al.*,[11] Dunstan and Read,[12] Makoshi,[13] and Schulze and Liebner.[14] It is generally accepted, however, that even if the two alkaloids are not identical they are at least isomeric.

Aconitine contains three hydroxyl groups and four methoxyl groups. When heated dry, it decomposes, yielding acetic acid. Hydrolysis can also be achieved by boiling the alkaloid with water under pressure or with a dilute acid. In both cases benzoylaconine is obtained together with acetic acid.

Benzoylaconine, also known as benzaconine, isaconitine, or picraconitine ($C_{32}H_{45}O_{10}N$, m.p.: 130°, α_D +5.6°), contains four free hydroxyl groups, but its tetra-acetyl derivative is not identical with the triacetyl derivative which can be obtained from aconitine.[15]

Aconine, $C_{25}H_{41}O_9N$, is obtained from aconitine by hydrolysis with alkali, whereby both acetic and benzoic acids are eliminated. It has

m.p.: 132° and α_D +23°. It contains four methoxyl and one ethylimino group,[16] and five hydroxyl groups, which can be acetylated (penta-acetyl aconine, m.p.: 232°).[17]

When aconitine is autoclaved with methanol, methylbenzoylaconine (m.p.: 211°) is formed by replacement of the acetyl group with methanol.[18] Heating of aconitine to its melting point leads to the formation of pyraconitine, $C_{32}H_{43}O_9N$,[19] which is also obtained from japaconitine by the same procedure. Hydrolysis of pyraconitine yields pyraconine, $C_{29}H_{39}O_8N$, and benzoic acid.

Oxidation of aconitine with potassium permanganate in acetone yields oxonitine, $C_{32}H_{43}O_{12}N$,[20] the ethylimino group being destroyed with the formation of acetaldehyde.[16] Oxidation of aconitine with potassium permanganate as well as oxidation with nitric acid leaves the carbon skeleton intact.[21] Oxonitine still contains the four methoxyl, the acetyl, and the benzoyl groups of aconitine, and it can be hydrolyzed with dilute alkali to oxonine, $C_{23}H_{37}O_{10}N$. The last forms a penta-acetyl derivative, which can also be obtained directly by oxidation of penta-acetyl aconine.[22] Oxonitine has four active hydrogen atoms and behaves as a lactame, suggesting the presence of an —CO—NH— group. Both oxonitine and aconitine have three double bonds, but aconine has none.

Oxidation of aconitine and oxonitine with fuming nitric acid yields the same nitroso-dicarboxylic acid, $C_{22}H_{26}O_{11}N_2$ (m.p.: 205°).[23] Lawson [24] obtained a nitronitroso derivative of aconitine by the action of dilute nitric acid, $C_{31}H_{35}O_{13}N_3$ (m.p.: 268°), which could be hydrolyzed to nitronitroso-aconitinic acid, $C_{22}H_{29}O_{13}N_3$.[25] If oxonitine is treated with nitric acid in the presence of acetic acid, a compound $C_{31}H_{35}O_{14}N_3$ (m.p.: 263°) is obtained.[24] Suginome [26] investigated the oxidation of aconitine, oxonitine, and mesaconitine with nitric acid, and found that all three gave rise to the same derivative, $C_{31}H_{33}O_{13}N_3$, which lost its solvent of crystallization at 180–205° and finally melted at 282°, but upon hydrolysis he obtained a compound (nitronitroso-aconinic acid), $C_{22}H_{27}O_{11}N_3$ (m.p. 298°). The following extended formula for the compound $C_{31}H_{35}O_{13}N_3$ has been suggested:

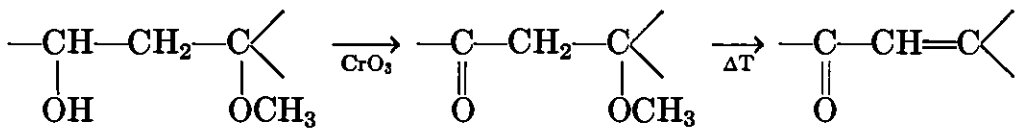
$$C_{18}H_{16}\begin{cases}(OCH_3)_3\\(OH)\\(OCOCH_3)\cdot\\(OCOC_6H_5)\\(NO_2)\\(=N—NO)\\(COOH)\end{cases}$$

This compound shows considerable similarity with an analogous one obtained from pseudaconitine:[27]

$$C_{18}H_{19}\begin{cases}(OCH_3)_3\\(OH)\\(CO)\\(OCOCH_3)\\(OCOC_6H_2(OCH_3)_2(NO_2)3,4,6)\\(NO_2)\\(=N—NO)\end{cases}$$

Oxidation of aconine with chromic acid gives rise to several compounds, among which is an amino-alcohol, $C_{24}H_{35}O_8N$, containing a tertiary nitrogen atom, four hydroxyl and three methoxyl groups, and the corresponding amino-acid,[28] $C_{24}H_{33}O_9N$.

When aconitine is oxidized with chromic acid, aconitoline is formed, which can be hydrolyzed further.[26] Majima and Tamura [21] have found that the primary product of that oxidation is aconitinone, $C_{34}H_{45}O_{11}N$, which then loses methanol and forms aconitoline, $C_{33}H_{41}O_{10}N$, which they now call desmethanol-aconitinone. These authors suggest the possibility of the presence of a carbonyl-beta-tertiary methoxyl group, which would easily lose methanol at higher temperatures, and they suggest the following mechanism:



Japaconitine. As stated previously, this alkaloid, $C_{34}H_{49}O_{11}N$, is identical or isomeric with aconitine (see also Pope [29] and Schwankte [30]).

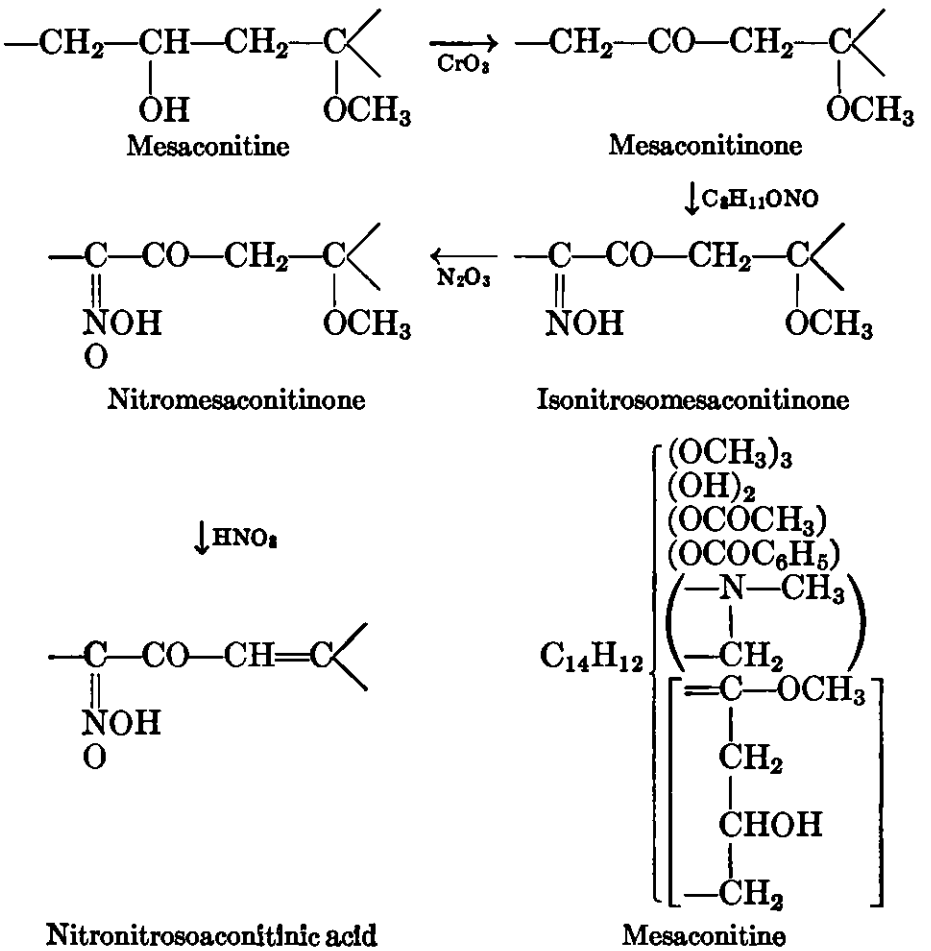
The base contains four methoxyl groups, forms a triacetyl derivative (m.p.: 166°), a methiodide (m.p.: 225°), and a methyl derivative (m.p.: 206°). When hydrolyzed, it yields at first benzoyljapaconine (m.p.: 183°), and then japaconine (m.p.: 100°), while, upon heating, pyrojapaconitine, $C_{32}H_{45}O_9N$ (m.p.: 168°), is produced. This last substance is said to be identical with pyraconitine.[14]

Mesaconitine. This base, $C_{33}H_{45}O_{11}N$, is said to be a lower homologue of aconitine,[31] with the ethylimino group replaced by a methylimino group.[32]

The alkaloid contains four methoxyl groups, yields a triacetyl derivative, and is hydrolyzed by dilute boiling acid to benzmesaconine (benzoylmesaconine), $C_{31}H_{43}O_{10}N$, which in turn can be hydrolyzed further to mesaconine (m.p.: 229°). When heated dry, the alkaloid loses acetic acid and forms pyromesaconitine, $C_{31}H_{41}O_9N$. Mesaconitine, when oxidized with potassium permanganate in acetone solution, yields oxonitine (m.p.: 282°), which is identical with the oxonitine obtained

from aconitine under similar conditions. According to Suginome,[33] the rate of oxidation of the two alkaloids differs. Mesaconitine decolorizes two moles of potassium permanganate very soon, but the action of the third mole of oxidizing agent takes somewhat longer. The yield of oxonitine is 62.4%. In the case of aconitine, four moles of potassium per manganate are required, the oxidation is appreciably slower, and the yield of oxonitine is only 30%.

When mesaconitine is oxidized with chromic acid, the ketone mesaconitinone ($C_{33}H_{43}O_{11}N$) is obtained.[34] This compound forms a semicarbazone, an oxime and a diacetyl derivative, and probably contains one enolized hydroxyl group. Hydrolysis furnishes benzmesaconinone first, which on heating loses methanol and water and forms desmethanol-anhydro-mesaconitinone. Desmethanol-mesaconitinone corresponds to aconitoline.[26] These authors suggest the possibility of a cyclohexanone ring, with possible enolization of the keto group. They also found that, of the three double bonds present in the molecule, only one hydrogenates easily, one adds water, and the third is very difficult to hydrogenate. They exclude the presence of an aromatic nucleus, and suggest the following mechanism and partial formula of mesaconitine.



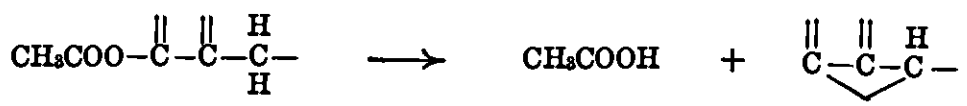
Jesaconitine. The alkaloid has been isolated by Makoshi [13] and by Majima and Mario,[31] and the formula $C_{35}H_{49}O_{12}N$ has been assigned to it. Hydrolysis of the base furnishes jesanisaconine, $C_{33}H_{47}O_{11}N$, which was found to be the para-anisoyl ester of aconine.[32] Permanganate oxidation of jesaconitine gives rise to acetaldehyde, implying the presence of an ethylimino group.[32] Also obtained as a product of the oxidative degradation of triacetyl jesaconitine by way of triacetyl-jesoxonitine is penta-acetyloxonine, which Tamura has also obtained from aconitine.

Hypaconitine. The base,[31,32] $C_{33}H_{45}O_{10}N$, was found to contain one methylimino and four methoxyl groups. Among its products of degradation are: benzhypaconine, $C_{31}H_{43}O_9N$; hypaconine, which forms a tetra-acetyl derivative (m.p. 184°), pyrohypaconitine (m.p.: 120°), and hypoxonitine (m.p.: 268°) obtained by permanganate oxidation.

Pseudaconitine. This alkaloid, $C_{36}H_{51}O_{12}N$, has been described by a number of investigators.[11,15,27,35] Hydrolysis of neutral solutions under pressure (135°) yields veratroylpseudaconine, $C_{34}H_{49}O_{11}N \cdot H_2O$ (m.p.: 199°), which can be hydrolyzed further to veratric acid and pseudaconine, $C_{25}H_{41}O_8N$, which crystallizes with solvent of crystallization. It has four hydroxyl groups and forms a tetra-acetyl derivative (m.p.: 228°).

Pseudaconitine contains six methoxyl groups, including the two of the veratric acid, and two hydroxyl groups (diacetyl derivative, m.p.: 229°).

It has been pointed out by Sharp [36] that one acetyl group is rather labile and can be replaced by methyl in both pseudaconine and its tetra-acetyl derivative by the action of methanol, or it can be eliminated by heating pseudaconitine, resulting in the formation of pyropseudaconitine. To explain this reaction, Sharp [36] has suggested the formation of a bridge, involving a somewhat removed carbon atom. He excluded the possibility of interaction with a hydroxyl group, since neither of the free hydroxyl groups is affected. A rearrangement involving the formation of a double bond was also discarded in favor of the first-mentioned theory.



In the analogous reaction in aconitine, the formation of an ether-linkage has been favored.[37] Acetylation of pyropseudaconitine furnishes triacetyl-demethyl-pyropseudaconitine, when one methoxyl group is replaced by acetoxy, and the two free hydroxyl groups are acetylated.

The oxidation of pseudaconitine with chromic or nitric acid has been investigated by Henry and Sharp.[27] The latter yields two compounds. One, $C_{34}H_{43}O_{17}N_3$ (m.p.: 195°), contains a nitrosamino group, and can be hydrolyzed, but only 6-nitroveratric and acetic acids have been identified. The other compound, $C_{33}H_{40}O_{16}N_4$ (m.p.: 260–270°), contains no alkylimino group, which has been replaced by a nitrosamino group, but it contains one acetyl, two nitro, and five methoxyl groups. This compound can be hydrolyzed to 6-nitroveratric acid and a residue, $C_{22}H_{31}O_{10}N_3$, containing the remaining nitro- and the nitrosamino group. The changes involved are thought to be as follows:

$$C_{18}H_{20}\begin{cases}(OCH_3)_3\\(OH)\\(OCOCH_3)\\\left(OCOC_6H_4(OCH_3)_2\ (3,4)\right)\\(CO)\\(N{-}CH_3)\end{cases} \xleftarrow{CrO_3} C_{19}H_{21}\begin{cases}(OCH_3)_4\\(CHOH)\\(OH)\\(OCOCH_3)\\\left(OCOC_6H_3(OCH_3)_2\ (3,4)\right)\\(N{-}CH_3)\end{cases}$$

$$\downarrow HNO_3$$

$$C_{18}H_{19}\begin{cases}(OCH_3)_3\\(CO)\\(OH)\\(OCOCH_3)\\(OCOC_6H_2(OCH_3)_2NO_2)\\(N{-}NO)\\(NO_2)\end{cases}$$

This N-methyl group could well be an N-ethyl group, in which case the hypothetical parent hydrocarbon would have the same empirical formula as the one of aconitine, $C_{20}H_{30}$.

Indaconitine, $C_{34}H_{47}O_{10}N$, contains four methoxyl groups. On hydrolysis it yields acetic acid and benzoylpseudaconine, and finally pseudaconine. The free base and its hydrochloride yield two different pyro-derivatives when heated, the former giving rise to alpha-pyro-indaconitine (m.p.: 133°), while the latter yields beta-pyroindaconitine (hydrobromide m.p. 250°).[38]

Bikhaconitine,[39] $C_{36}H_{51}O_{11}N \cdot H_2O$, contains six methoxyl groups, two of which are in the veratric acid residue obtained upon hydrolysis. The intermediate product of hydrolysis is veratroylbikhaconine ($C_{34}H_{49}O_{10}N$, m.p.: 125°), and the final product is bikhaconine, $C_{25}H_{41}O_7N$, which is soluble in ether, while aconine, pseudaconine,

and japaconine are only sparingly soluble in that solvent. Pyrobikhaconitine, $C_{34}H_{47}O_9N$, is formed at 180°.

Lappaconitine, as well as the following two alkaloids, has been isolated by Rosendahl,[40] by Schulze and Ulfert,[41] and by Weidmann.[42] Data obtained for these bases differ greatly. The empirical formula of lappaconitine is $C_{32}H_{44}O_8N_2$ or $C_{32}H_{42}O_9N_2$. It contains a methylimino and either two or three methoxyl groups. Its products of hydrolysis are picrolappaconine (anthranoyllappaconine) and lappaconine, also acetic and anthranilic acids, although acetyl-anthranilic acid has been obtained as the sole product of hydrolysis.[42]

Septentrionaline, $C_{33}H_{46}O_9N_2$, and *Cynoctonine*, $C_{36}H_{55}O_{13}N_2$, have been found together with the above alkaloid. The former base (m.p.: 131°) contains four methoxyl groups. Hydrolysis gives rise to an amorphous base, $C_{25}H_{39}O_7N$, and a homologue of anthranilic acid.

Lycaconitine and *Myoctonine*, $C_{36}H_{46}O_{10}N_2$ and $C_{36}H_{42}O_{10}N_2$ respectively, have been described by Schulze and Bierling,[43] Dragendorff and Spohn,[44] and others.[45] Among the decomposition products of both alkaloids are succinic and succinylanthranilic acids.

Minor alkaloids accompanying aconitine are neopelline and napelline. The former, $C_{32}H_{45}O_4N \cdot 3H_2O$, contains three methoxyl and methylimino group, and on hydrolysis it gives rise to acetic and benzoic acids and to neoline,[46] $C_{23}H_{39}O_6N$. Napelline, $C_{22}H_{33}O_3N$, on dehydrogenation with selenium, yields a hydrocarbon, $C_{17}H_{16}$, which is thought to be identical with one obtained under similar procedure from atisine.[47]

The Atisines

This group of alkaloids, which are polyhydric amino-alcohols, often accompany and may even predominate the true aconitines. They are thought to be structurally related to the aconines. All but one (heteratisine) are thought to be unsaturated, but the existence of some hydrogenation-resisting unsaturation is not ruled out.[48] The aconines are thought to have 19 carbons exclusive of methoxyl and N-alkyl groups; heteratisine, hetisine, and kobusine may have a 20-carbon skeleton; atisine, napelline, and staphisine may have a 21-carbon skeleton, unless the N-alkyl group is an ethyl group, which would reduce the hydrocarbon to 20 carbons and make possible a diterpene structure.[48]

Atisine. This base has been described by Broughton,[49] Wasowicz,[50] Wright,[51] Jowett,[52] and Lawson and Topps,[53] who confirmed the empirical formula $C_{22}H_{33}O_2N$.

Jacobs and Craig [54] found atisine to contain one N-methyl group but no methyl groups, but no methylenedioxy group could be found. Atisine, as well as its tetrahydro-derivative, contains two active hydrogens

in the form of hydroxyl groups, which can be acetylated. The alkaloid is unstable in an alkaline solution. A rearrangement of its double bonds is thought to be the cause of this, because the rearrangement does not occur in the tetrahydro-derivative. Under the action of alkalies a demethylated base is obtained from atisine, which still retains its N-methyl group ($C_{21}H_{31}O_2N$, m.p.: 147°). Atisine forms a dihydro-derivative, $C_{22}H_{35}O_2N$, which can be hydrogenated further to the tetrahydro-derivative (m.p.: 171–174°). An isomeric tetrahydro-derivative has been obtained by the hydrogenation of atisine with platinum oxide.

Dehydrogenation of atisine with selenium yields a variety of hydrocarbons and nitrogen-containing bases.[53] Among the hydrocarbons, 1-methyl-phenanthrene has been definitely identified.[55] The carbon skeleton of atisine is thought to have a pentacyclic structure.[54]

Lucidusculine,[56] $C_{24}H_{37}O_4N$, is thought to be related to atisine. The base yields a mono-acetyl-derivative and can be hydrolyzed to acetic acid and luciculine, $C_{22}H_{35}O_3N$.

Staphisine $C_{22}H_{31}ON$?, has been isolated by Jacobs and Craig [57] from *Delphinium staphisogria* seeds, where it was found in the motherliquors from the preparation of delphinine.[48] Staphisine contains an N-methyl but no methoxyl group. The oxygen might be present in the form of an ether. There are also two conjugated double bonds. Dehydrogenation yields a number of multi-methyl-derivatives of phenanthrene. Among its dehydrogenation products is 1,7-dimethyl-phenanthrene.[55] The products of oxidation have also been examined by the same authors.

Heteratisine, $C_{22}H_{33}O_5N$, and *hetisine* have also been isolated by Jacobs and Craig.[58] The former contains one methoxyl group and one N-methyl group. It is a lactone, which can be split under the influence of alkalies and relactonized with concentrated hydrochloric acid. There are no double bonds susceptible to hydrogenation. Hetisine contains no N-methyl and no methoxyl group, but it has three active hydrogens possibly in the form of hydroxyl groups. It contains at least one double bond.

Kobusine, $C_{20}H_{27}O_2N$ (m.p.: 268°), has been found in the motherliquors of jesaconitine.[59] It is a tertiary base, containing no acetyl, benzoyl, methoxyl, or methylimide group. It gives no carbonyl reaction, no derivative with diazomethane, and no nitrosamine with nitrous acid. It does, however, form a diacetyl-derivative and contains two double bonds, one of which is on the nitrogen atom, because tetrahydrokobusine forms a triacetyl-derivative.

Recently four new bases have been isolated from *Aconitum talassicum* by Konovalova and Orekhov.[60]

TABLE 3.1

		Aconitine	Aconine	Japaconitine	Indaconitine	Mesaconitine	Hypaconitine	Pseudaconitine	Bikhaconitine	Atisine
Base	m.p.	198	132	205	203	209	198	213	118	(85?)
	α_D	+14.6 +18.7	+23	+20.3	+18.3	+25.7	+22.7	+17 +22.7	+12.2	−14.6
HBr	m.p.	207	225	173	187		179	199	175	273
	H_2O *	2½, ½	1½	4			2½	3	5	
	α_D	−30.8			−17.3			−18.5	−12.4	+24.3
HCl	m.p.	195	176	150	171			182	161	296
	H_2O *	3½	2	3				4	5	
	α_D	−30.9	−7.7	−23.8				−18.1	−8.8	+18.5
HI m.p.		226		210				230	194	280
Aurichloride m.p.		176 †		160 231	152	226		233	233	
Perchlorate m.p.		222				225	180	239		
Acetyl-derivative		208	232	166			245			
Nitrate m.p.		199		177 194	203			198	180	252

* H_2O of crystallization.
† Also 152, 135.

NATURAL SOURCES

The genus Aconitum comprises about sixty species, not counting a number of sub-species, most of which are poisonous, although some have been classified as nonpoisonous. While only the dried root of *Aconitum napellus* Linné of the family Ranunculaceae is official in the United States, many other aconite species have been used in medicine.

Commercial aconite has its origin from plants of varieties or subspecies of *Aconitum napellus*, which grow wild in the mountainous regions of Central Europe and Spain, although the plant is also found in Eastern Europe and in Siberia as far as the Himalaya Mountains. The plant is also found in North America where it has been cultivated. Its native and very close counterpart is *Aconitum delphinifolium*, which is found in western and central Canada. Some twenty odd species of Aconitum are indigenous to the United States, but most of them are found in the western and mountainous parts of the country.

The plant is a perennial herb, 2–5 ft high. Underground is found a conical or fusiform tuberous root, which reaches a maximum of about 4 in. in length and about 1 in. in width at the top. The tubers are brownish outside and whitish and fleshy inside, and they send out numerous thick rootlets. One or more of the lateral shoots usually form a conical daughter tuber, and in the full-grown plant two or sometimes three tubers are found joined together. The younger and smoother

root is often connected to the older and wrinkled root through a side branch of the latter. The young root usually furnishes the stem for the new plant in the following year of growth, at which time the old tuber dies and disappears. The stem is usually simple, erect, round, and smooth. The alternate leaves are petiolate, on long stalks. The blades are deep green on the upper surface and lighter green on the lower surface, and they are divided into three to five segments, which in turn are subdivided. The inflorescence is a terminal raceme, simple, straight, and erect, and of dark violet blue color. Smaller racemes grow from the axils of the upper leaves. The fruit is a three-to-four-beaked follicle, containing many wrinkled, angular seeds. The seeds lose their germinating power upon drying and storing for some time.

Commercial supplies originate from Germany, France, Switzerland, and Spain, where they are gathered from wild-growing plants, although extensive cultivations have been undertaken. Propagation is usually effected from seeds or by division of the roots, but the latter method is preferred to avoid hybridization. Seeds are planted almost as soon as gathered. Best conditions for cultivation are a shady, well-drained ground of gravel and loam, a cool climate, and considerable elevation above sea level. Roots of first-year plants are usually low in alkaloid content, but the tubers of older plants are dug up in the autumn or winter, after the leaves have fallen. They are washed, freed from rootlets, and are carefully dried.

The tubers, a full description of which will be found in the *National Formulary* (8th Edition), and in the *British Pharmacopœia*, occur in commerce in many different forms, some of which are as follows: single tubers, either smooth and light brown or darker and longitudinally wrinkled; twin tubers, where the parent tuber shows remains of a stem and the daughter tuber the remains of a bud; and single tubers which might be very small, very resinous, or almost cylindrical (their usual shape is conical).

Substitutes and adulterants are often found in commercial supplies, but they can usually be detected by visual examination without recourse to chemical analysis and identification of the alkaloids, which often are not aconitines.

Roots of about 1 in. length, little wrinkled, most of the rootlets clustered at the pointed (basal) end: Tubers of *Aconitum chasmanthum* Stapf; contains indaconitine.

Tubers of about the same dimensions as those from *Aconitum napellus*, but of grayish-brown color and very deeply wrinkled: Tubers from *Aconitum ferox* Wallich; contain pseudaconitine.

Tubers up to 6 in. long and almost 2 in. thick, also deeply wrinkled;

fracture horny instead of mealy: Indian or Nepaul Aconite (*Aconitum deinorrhizum* Stapf or *Aconitum balfourii* Stapf?); contains pseudaconitine.

Conical tubers, smaller and smoother, very mealy and of somewhat lighter specific gravity: possibly from *Aconitum fischeri* Reich; contains jesaconitine.

Less wrinkled and smaller, daughter root grayish, parent root brownish and somewhat raised in comparison with daughter root, distinct longitudinal ridges: a Japanese variety of Aconitum; contains japaconitine.

A list of the more important Aconitum species will be found in Table 3.2. A histological description of some of them can be found in the *U. S. Dispensatory.*

TABLE 3.2 NATURAL DRUGS

Drug	*Alkaloidal Content*	*Yield, %*
A. napellus Linné.....	aconitine, aconine, neopelline, napelline, *l*-ephedrine & sparteine	0.5
A. deinorrhizum Stapf	pseudaconitine	0.7
A. spicatum Stapf.....	bikhaconitine	0.6
A. balfourii Stapf.....	pseudaconitine	0.4
A. chasmanthum Stapf.....	indaconitine	
A. calliantum Koidzumi....	hypaconitine, mesaconitine, traces aconitine	0.3
A. grossedentatum Nakai...	aconitine, hypaconitine, mesaconitine	0.4
A. hacusanense Nakai.....	aconitine, hypaconitine, mesaconitine	0.4
A. ibukiense Nakai.....	hypaconitine, mesaconitine, aconitine	0.3
A. kamtschaticum Willd & Reichb	hypaconitine	0.2
A. majima Nakai.....	aconitine, mesaconitine	0.23
A. sachalinense Fr. Schmidt	jesaconitine, aconitine	0.3
A. stoerkianum Reichb.....	neopelline, aconitine?	
A. subcuneatum Nakai.....	aconitine, jesaconitine	0.5–0.7
A. tortuosum Willd.....	hypaconitine, aconitine	0.3
A. zuccarini Nakai.....	aconitine, mesaconitine	0.15

MANUFACTURING METHODS

The aconite alkaloids can be obtained in commerce in the form of tinctures, fluid extracts, and amorphous and crystalline alkaloids. The preparation of the tincture and the fluid extract are described in the *National Formulary* (8th Edition), and nothing further need be said in reference to these two preparations. Amorphous aconitine is a crude mixture of the total alkaloids of aconitum together with a considerable amount of resinous impurities. This material may contain as little as

3% of crystalline aconitine, and its manufacture does not present any special problems and will not be discussed here. The commercial production of crystalline aconitine and the isolation of the main alkaloids from certain other species will be described.

The Manufacture of Aconitine

Aconitine is usually prepared from *Aconitum napellus*, which is reduced to a medium powder (40-mesh) prior to extraction. Because of the relative scarcity of the drug, its high price, and the rather delicate nature of the alkaloid, batches should be kept rather small and should not exceed 100 lb. Almost any iron, monel-metal, or stainless steel percolator or extractor can be used, with the last-mentioned to be preferred. Copper extractors should not be used; wooden barrels have been used with much success.

The crude drug is now extracted with dry methanol, in which 1% of tartaric acid had been dissolved previously. The extract should be circulated and, if possible, heated to about 30°. Higher temperatures should be avoided. After 12 hr, the extract can be drained off, and it should then be put aside. The crude drug is extracted three times more with dry methanol containing 1% of tartaric acid. The second, third, and fourth extracts are concentrated under vacuum, with continuous stirring, and with the temperature not exceeding 30°. When the concentration is nearly finished, the first extraction is added and concentrated. In this way extensive heating of the main amount of the alkaloids is avoided.

The thick syrupy residue is thoroughly mixed with five to ten times the amount of water. If difficulties are experienced in obtaining a more or less homogeneous mixture, some petroleum ether or a small amount of carbon tetrachloride can be added to dissolve some of the resinous material. The aqueous phase should be washed twice more with petroleum ether to remove all fatty impurities. The petroleum ether washings, together with any carbon tetrachloride, are combined and are re-extracted twice with dilute (1%) hydrochloric acid or sulfuric acid. These washings are recombined with the original aqueous (tartaric) acid solution.

The total aqueous liquor is now made alkaline with either ammonia or potassium carbonate, and the total alkaloids are extracted at once with ethyl or isopropyl ether or, preferably, with benzol or toluol. Using the first two solvents, a cleaner product will be obtained immediately, but because of the lower solubility of aconitine more extractions will be required. Emulsions should be broken at once by filtration with filteraid. Somewhat speedy operation is necessary throughout the whole

process. The aqueous phase can be discarded when Mayer's reagent yields only a cloudiness after acidification of a sample.

The combined benzol or toluol extracts can be washed once with water if a preliminary test indicates that no emulsion will be formed. The solvent is then extracted with small amounts of hydrobromic acid (36%), until a slight acid reaction to congo test paper is obtained. Aconitine hydrobromide is allowed to crystallize, and crystallization can be aided by the addition of small amounts of ammonium bromide. Slimy precipitations can be brought to crystallization, after the main liquor has been decanted, by mixing with small amounts of isopropanol or acetone. After the aconitine hydrobromide has been allowed to crystallize in the cold for 24 hr, it is filtered off and washed with acetone. The motherliquors are made alkaline (cool with ice), extracted with benzol, and further amounts of aconitine can then be obtained in similar fashion as before. The crude aconitine hydrobromide is then recrystallized from alcohol (methanol or isopropanol) and acetone (or methyl ethyl ketone), after slight warming (35°), and treating with charcoal. The salt, after drying, should have a melting point of 206–207°. When recrystallizing from alcohol, crystallization can often be induced by adding some ether and a small amount of distilled water to furnish water of crystallization.

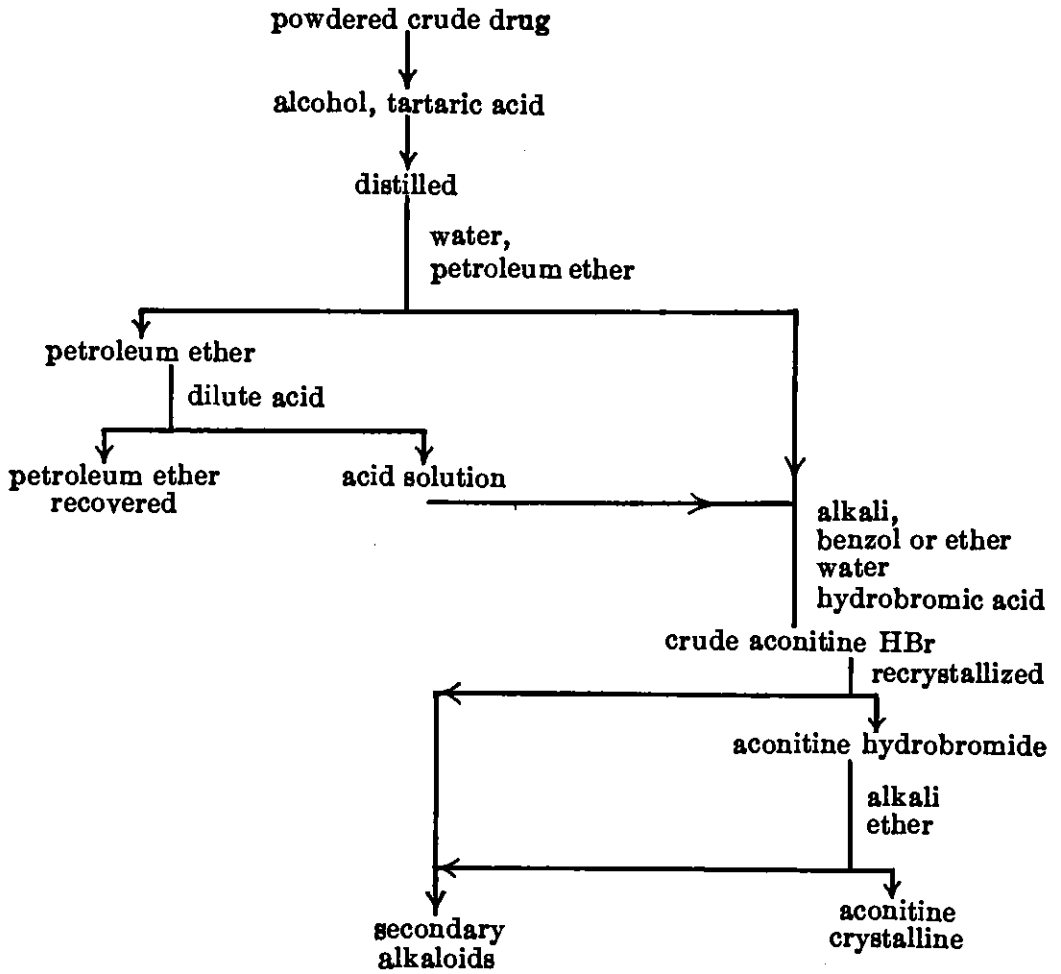
An interesting variation of the foregoing method of purification is the following one, which has been used successfully. Instead of extracting the solvent phase with hydrobromic acid, it is extracted with 10% hydrochloric acid until the solution is slightly acid to congo test paper. To this solution is then added a very concentrated solution of sodium iodide until precipitation of aconitine hydriodide is complete. After recrystallization from water, the salt shows a melting point of 226°.

With the alkaloid thus purified, it is relatively easy to prepare the free base or any other salt wanted in a pure state. To prepare the free base, the salt is mixed with water and benzol. The alkaloid is liberated with ammonia, and stirring is continued until all the alkaloid is dissolved. The benzol is separated, dried thoroughly with anhydrous sodium sulfate, concentrated at a low temperature (30°) to a small volume, and the aconitine base is crystallized on the addition of ether. It can be recrystallized from alcohol. Other salts can be prepared from the final benzol solution as desired.

According to another method of manufacture, which also gives good results, the crude powdered drug is extracted with a mixture of three parts of methanol and two parts of amyl alcohol or one part of a butyl alcohol, somewhat acidified with tartaric acid. The combined extracts are distilled under vacuum until a rise in the temperature indicates that

all the methanol has been removed and the amyl or butyl alcohol solution of aconitine remains in the still. The total alkaloids are then removed by repeated extraction with dilute hydrochloric acid. The aqueous acid solution is washed once or twice with petroleum ether. The alkaloids are then liberated with ammonia, extracted with benzol, and are purified further as previously described.

If desired, the secondary alkaloids of Aconitum can be obtained from the motherliquors from the crystallization and recrystallization of aconitine hydrobromide or hydriodide, and of the base.



The Preparation of Japaconitine

This method is based on the procedure of Majima *et al.*[61] The crude, powdered drug is thoroughly mixed with 3% of its weight of calcium carbonate and is then extracted three times with 95% ethanol (denatured). The combined extracts are evaporated under vacuum below 30°. The residue is mixed with water, and resinous and fatty matter is removed by repeated extraction with petroleum ether. The petroleum

ether in turn is extracted several times with dilute sulfuric acid, which is then added to the original aqueous solution. Some japaconitine is precipitated by neutralization with sodium carbonate and can be filtered off. The motherliquor is made strongly alkaline with sodium carbonate and is then extracted with chloroform after the addition of some ammonium sulfate. When this chloroform solution is treated with dilute hydrochloric acid, secondary alkaloids are removed but japaconitine hydrochloride remains dissolved in the chloroform. After evaporation of the latter, the base is liberated from its salts in the usual manner, combined with the crude base obtained previously, and recrystallized similar to aconitine.

Other aconitine alkaloids are prepared by similar methods, but for further details the original literature should be consulted.

The Preparation of Atisine

The following method, recommended by Jacobs and Craig,[54] gives excellent results:

The ground root of *Aconitum heterophyllum* is extracted twice with 70% alcohol, and each time the extract is completely removed in a press. The combined extracts are concentrated under vacuum, the residue is treated with ¼ of its volume of 10% sulfuric acid, extracted with chloroform several times. The liquid is then made alkaline and extracted with benzol several times, which is distilled under vacuum after drying. The alkaloid is then crystallized from the residue with alcohol, and is finally purified by crystallization of the hydrochloride from 50% alcohol.

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

Aconitine, when administered locally, at first irritates and later paralyzes the peripheral endings of the sensory nerves. The same symptoms may even be observed after the injection of somewhat larger doses. When applied to the tongue, an intense tingling sensation is produced, which may be followed by numbness. Following systemic ingestion, tingling and smarting of the skin are experienced which is followed by cutaneous insensitivity.

Injection of aconitine causes a slowing of the pulse, lowering of the blood pressure and body temperature, and slower respiration. The heart beat becomes increasingly irregular, owing to excitation of the heteropic impulses,[62] and with large doses passes into fibrillation. The toxic effects of calcium and digitalis on the heart are increased by aconitine. Aconitine is extremely toxic, but varying lethal dosages have been recorded by different workers.[63]

Aconitine has been used to reduce overactivity of the heart, reducing the blood pressure and encouraging sweating. Because of its locally paralyzing effect it has been used in peripheral neuralgias or as a local anesthetic in the stomach. However, because of the closeness of the effective and lethal dose, its use has been largely discontinued. The toxicology of aconitine has been extensively described [64] and need not be discussed further.

The action of the other aconitines is similar in their action but different in their respective intensities. Japaconitine [65] and indaconitine [66] are more active than aconitine; bikhaconitine is somewhat more active than the latter, but less active than pseudaconitine,[66] which is said to be very active.[66] Jesaconitine [67] and mesaconitine [68] resemble japaconitine. Lycaconitine and myoctonine [69] and lappaconitine are weaker than aconitine. Atisine and the alkaloids of its group are almost nontoxic.[70] Methylbenzoylaconine is reported to have a curare-like action.[71]

ANALYTICAL AND TOXICOLOGICAL METHODS

Salts of aconitine, when in very concentrated solution, give precipitates with the following reagents: Platinic chloride, potassium chromate, mercuric chloride, potassium ferri- and ferrocyanide. But the following reagents yield precipitates even in dilute solutions: Potassium permanganate, red crystalline precipitate; picric acid and aurichloride yellow precipitates, the latter 1:5000; Mayer's reagent, a yellowish white precipitate in 1:10,000; phosphotungstic and phosphomolybdic acids give white precipitates; iodine solution gives a red-brown precipitate in 1:20,000.

Concentrated nitric and sulfuric acids give no color, but the latter, with a crystal of ammonium vanadate, gives an orange color. Heating with sulfuric acid and adding some resorcinol after a few minutes gives rise to an intense red color.

Aconitine can usually be found in the urine, the liver, the blood, and the stomach, provided there is no alkalinity present, in which case the aconite alkaloids are destroyed. Stomach and intestines should be examined for remains of aconitum plants. For the detection of the alkaloids the organs are extracted with acidified alcohol which is then strained off, evaporated under vacuum, and the alkaloids are determined chemically or biologically.

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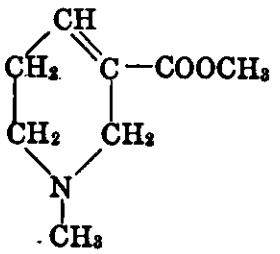
CHAPTER IV

ARECOLINE AND RELATED ALKALOIDS

CHEMISTRY

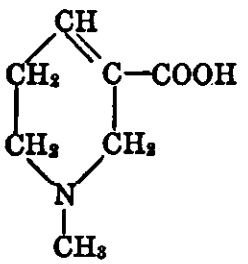
Although the fruit of the betel palm and the leaves of the betel pepper are used by many millions of people as a masticatory, the arecoline derived from the fruit is used only in veterinary medicine.

Arecoline is the main and physiologically most active alkaloid. Its formula has been established as N-methyl-2,5,6-tetrahydropyridine-3-carboxy-methyl ester:



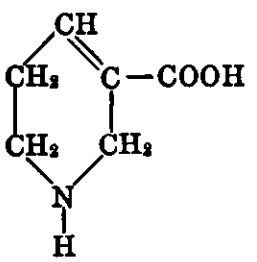
Arecoline

Its structure has been ascertained by independent synthesis as well as by its relation to the other alkaloids of this group. It was found that arecoline is the methyl ester of arecaidine:



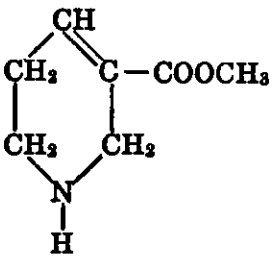
Arecaidine

which, in turn, was shown to be N-methyl-guvacine:



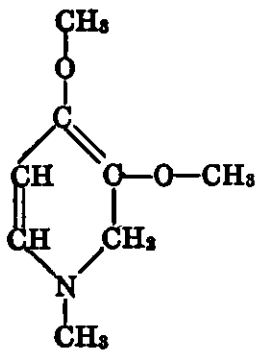
Guvacine

Guvacoline is the methyl ester of guvacine or nor-arecoline. By the same token guvacine is nor-arecaidine.



Guvacoline

Isoguvacine, which for a time was thought to be a separate alkaloid, is now assumed to be identical with arecaidine. Arecolidine, however, has been isolated and the following formula has been temporarily assigned to it, which is that of 3,4-dimethoxy-1-methyl-1,2-dihydropyridine.[1]



Arecolidine

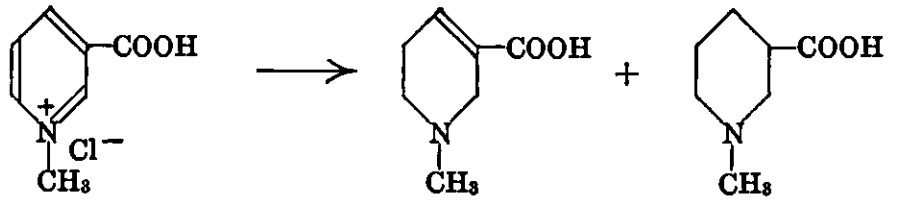
Guvacine, the simplest of the bases, was found to react as a secondary amine, giving rise to a nitroso compound (m.p.: 168°) and an acetyl derivative (m.p.: 190°).[2] On distillation with zinc dust it gives beta-picoline.

Trier suggested [3] and Freudenberg proved [4] the identity of guvacine with Δ^3-tetrahydropyridine-3-carboxylic acid, which had previously been synthesized by Wohl and Losanitsch.[5] Freudenberg [4] also showed that esterification with methanol gives guvacoline.

Guvacoline, in turn, on treatment with methyl iodide gives a mixture of arecoline methiodide and arecoline hydriodide. Arecoline, on the other hand, was shown to yield arecaidine and methanol upon hydrolysis. Arecaidine was thus found to have a carboxy group, but it also proved to be amphoteric in character, forming salts with both acids and alkalies. The nitrogen atom was found to carry a methyl group, which could be removed as methyl chloride by the action of hydrochloric acid at 240°. Methylamine was formed on treatment with lime.

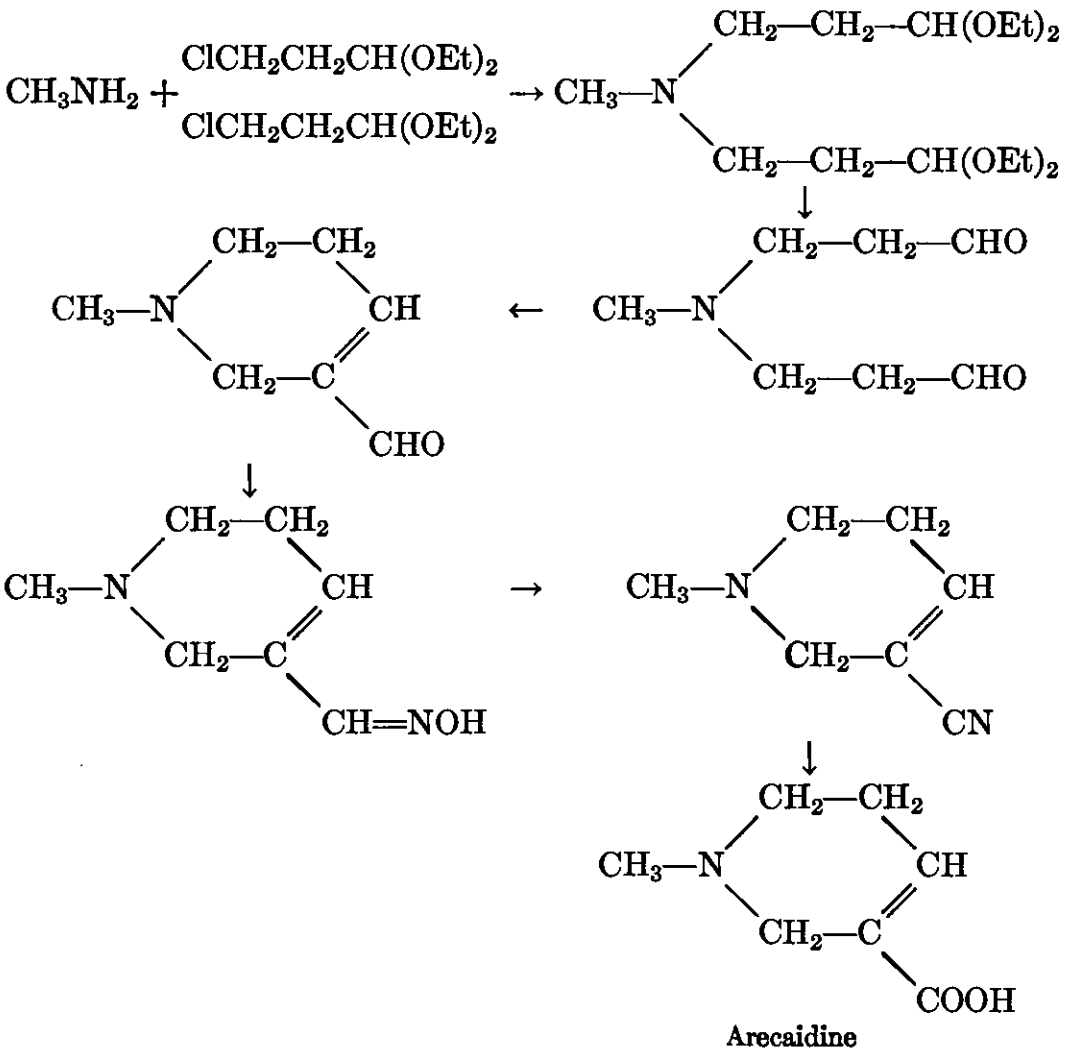
The relation of arecaidine to nicotinic acid was established when the

methochloride of the latter, on reduction with tin and hydrochloric acid, gave arecaidine and dihydroarecaidine.



The aforementioned evidence proved conclusively the identity of arecoline as that of a nicotinic acid derivative, but the position of the double bond was yet to be determined. Because of the optical inactivity of arecaidine, only positions 2,3 or 3,4 could be considered. The location of the double bond was finally determined by complete synthesis of arecoline or arecaidine.

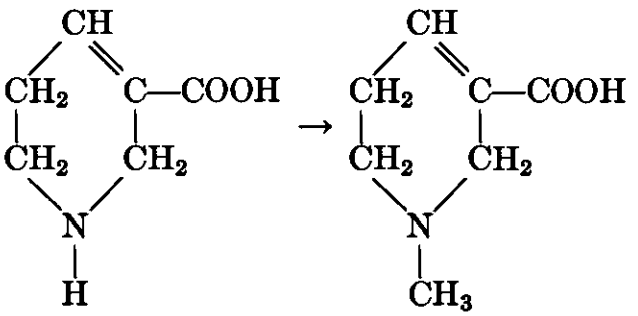
According to the method of Wohl and Johnson,[6] beta-methyliminodipropionaldehyde diacetal was obtained by the condensation of beta-



Arecaidine

chloropropionaldehyde acetal with methylamine, the first having been obtained by the action of alcohol and hydrogen chloride on acrolein. Hydrolysis of the diacetal followed by ring closure gave the aldehyde corresponding to arecaidine, having the double bond in the 3,4-position. Arecaidine was obtained from the aldehyde by way of the oxime and the cyano-compound.

The constitution of guvacine as nor-arecaidine was established when it was transformed into arecaidine by N-methylation.[2]



Isoguvacine, discovered by Jahns,[2] is of doubtful constitution but is now assumed to be mainly arecaidine. On distillation with zinc dust, however, it yields a substance which gives a strong pyrrole reaction, thus giving rise to the hypothesis that it is a simple pyrrole derivative, isomeric with guvacine. Trier,[3] on the other hand, thought it to be Δ^2-tetrahydropyridine-3-carboxylic acid.

Guvacine, $C_6H_9O_2N$, crystallizes in small, lustrous prisms or scales. It is soluble in dilute alcohol, slightly soluble in water, and practically insoluble in other organic solvents. The base is optically inactive and neutral to litmus; the salts are crystalline and react slightly acid. The hydrochloride, nitrate, aurichloride, and platinichloride crystallize in prisms of different shape, but the sulfate crystallizes in scales. All salts decompose at their melting points.

Guvacoline, $C_7H_{11}O_2N$, is a colorless oil, but the salts are crystalline. The hydrobromide crystallizes in prisms; most other salts and especially the hydrochloride and platinichloride crystallize in scales.

Arecaidine, $C_7H_{11}O_2N$, crystallizes from 60% alcohol as the monohydrate, which will lose the water of crystallization at 100° only. The monohydrate crystallizes as hexagonal or quadratic plates. The base is soluble in water and dilute alcohol, slightly soluble in absolute alcohol, and insoluble in other organic solvents. The hydrochloride crystallizes from absolute alcohol in needles.

Arecoline, $C_8H_{13}O_2N$, is an odorless, colorless oil of strongly basic character, soluble in water and most organic solvents, and volatile with steam. The salts are deliquescent.

Arecolidine, $C_8H_{13}O_2N$, crystallizes in hygroscopic needles and can be sublimed. The aurichloride crystallizes in leaflets and the platinichloride in needles. The base is easily soluble in water and organic solvents and has a characteristic basic odor.

TABLE 4.1

	Arecoline	Arecaidine	Guvacoline	Guvacine	Isoguvacine	Arecolidine
Base m.p.....	.....	233	.....	295	220	110°
Base b.p.....	210–215	...	114/13 mm	...	...	....
Hydrochloride..	158	261	122	316	231	250
Hydrobromide..	172		145			272
Aurichloride....	oil	199		199	200	220
Platinichloride..	176	226	211	233	235	223

NATURAL SOURCES

"Areca is the dried ripe seed of *Areca catechu* Linné (Fam. Palmae)" (*National Formulary*, 8th Edition). The tree, indigenous to the Malay Archipelago, is now being cultivated in the Philippine Islands, India, China, East Africa, and it also has been naturalized in the American tropics.

The tree is a feather palm, with a tall, thin, unbranched stem, being topped by a large crown consisting of pinnate leaves with many leaflets up to 2 ft long. The fruit is an orange-yellow colored ovoid nut, containing in its fleshy interior a single seed with a small embryo and a large endosperm. To prepare them for shipment, the seeds are separated from the fruits, boiled in water containing some lime, and are then dried. They are packed in bags and are usually exported from Colombo, Ceylon, Padong, or Penang.

The crude drug is described in the *National Formulary* (8th Edition) as follows: "Rounded—conical, up to 3 cm in length and 2 cm in diameter; each seed up to 5 grams in weight; externally weak reddish brown to light yellowish brown, marked with a network of paler lines, and frequently with adhering portions of silvery brittle endocarp and adhering

fibers of the mesocarp at the base of the seed. Seed hard, the cut surface exhibiting a marbled appearance (ruminate endosperm) of brownish tissue alternating with whitish tissue.

"Areca contains not more than 2% of adhering pericarp and not more than 1% of other foreign organic matter, and yields not less than 0.35% of ether soluble alkaloids calculated as arecoline and not more than 2.5% of total ash."

Besides the several alkaloids (total alkaloids up to 0.45%, arecoline up to 0.2%), areca nut contains tannin, fat, fixed and volatile oils, and a small amount of gum.

The assay of Areca is described in the *National Formulary* (8th Edition), which also contains as official preparations arecoline hydrobromide and arecoline hydrobromide tablets.

MANUFACTURING METHODS

Methods for manufacturing the areca alkaloids have been published by Jahns [2] and by Chemnitius,[7] but neither can be recommended for large-scale manufacture. For that matter, the production of arecoline hydrobromide from the natural drug is almost as difficult and expensive as the large-scale synthesis of the alkaloid.

Jahns recommended digestion of the finely ground seed with acidified water (2% sulfuric acid), concentrating the clear extract and precipitating the total alkaloids by the addition of potassium bismuth iodide. The precipitate was filtered off, washed, and was then boiled with barium carbonate to liberate the alkaloids which were taken up with ether. From this ether phase arecoline was crystallized as the hydrobromide. The aqueous solution was neutralized with sulfuric acid, treated with silver sulfate, and the precipitate was filtered off. The filtrate was treated with barium hydroxide and sufficient carbon dioxide to precipitate the barium. The filtrate from this precipitate was evaporated to dryness and the residue extracted with alcohol or chloroform, leaving the arecaidine undissolved.

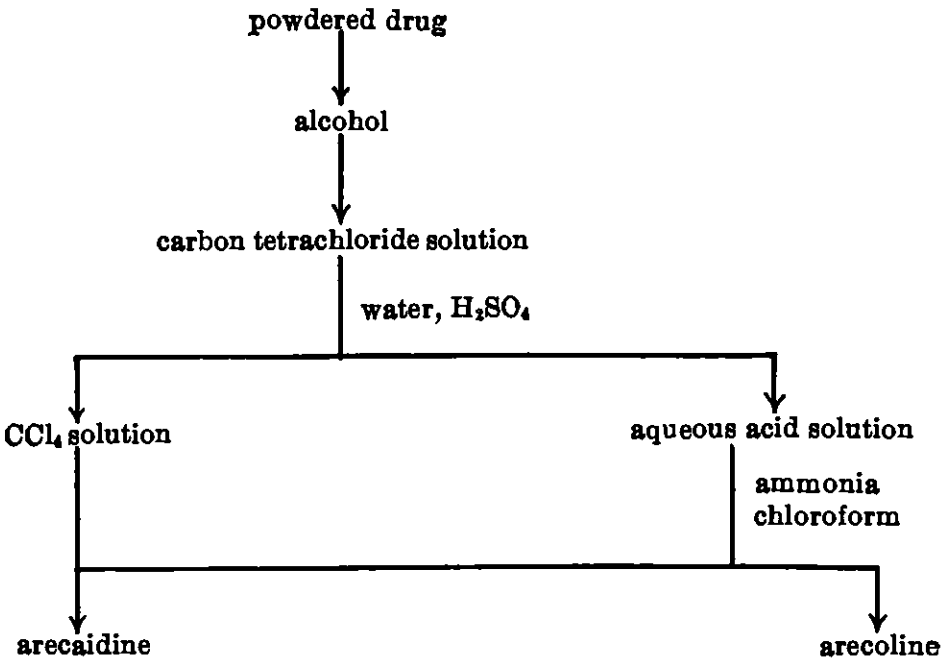
According to Chemnitius the powdered Areca seeds are thoroughly moistened with a 10% solution of potassium carbonate and are then extracted with hot ether three times. The ether extracts are combined and are allowed to cool. The aqueous phase which separates on cooling is put aside and is worked up separately because of impurities. The ether phase is mixed with 50% acetic acid and from the acid mixture the ether is removed completely by distillation. After cooling, fat and other impurities can be removed in solid form. The aqueous acid phase is now made alkaline with potassium carbonate, the alkaloids are ex-

tracted with ether, and arecoline is crystallized as the hydrobromide. From the aqueous phase put aside previously, the alkaloids are recovered in a similar manner, but arecoline is further purified by crystallization from alcohol before it is turned into the hydrobromide.

Excellent results have been obtained by the following method, which, however, serves to produce arecoline only. If extraction of the total alkaloids is desired for the purpose of transforming the secondary alkaloids into arecoline, Jahns' method of extraction is recommended.

The powdered drug is extracted with alcohol in the usual manner, and the alcohol is evaporated under vacuum. The syrupy residue is dissolved in carbon tetrachloride and is extracted several times with dilute sulfuric acid. This treatment removes the arecoline, but leaves the arecaidine dissolved in the organic solvent. The aqueous acid phase is collected, made alkaline, and the arecoline is removed by extraction with chloroform, six to seven extractions usually being necessary. The chloroform extracts are combined, dried thoroughly with sodium sulfate, and are distilled under vacuum to a thick syrup, which is almost pure arecoline base. Purification is best undertaken by recrystallization of the hydrobromide, which is prepared by adding 48% hydrobromic acid under stirring and cooling to a solution of the base in acetone.

Occasionally it has been possible to separate the arecaidine also by using the foregoing process described. Persistent emulsions, which interfere with extraction, are a main difficulty, which cannot always be remedied by filtration. The ammoniacal motherliquors (aqueous phase) from the chloroform extraction are used to extract the carbon tetrachloride solution, thus removing the arecaidine and other acidic



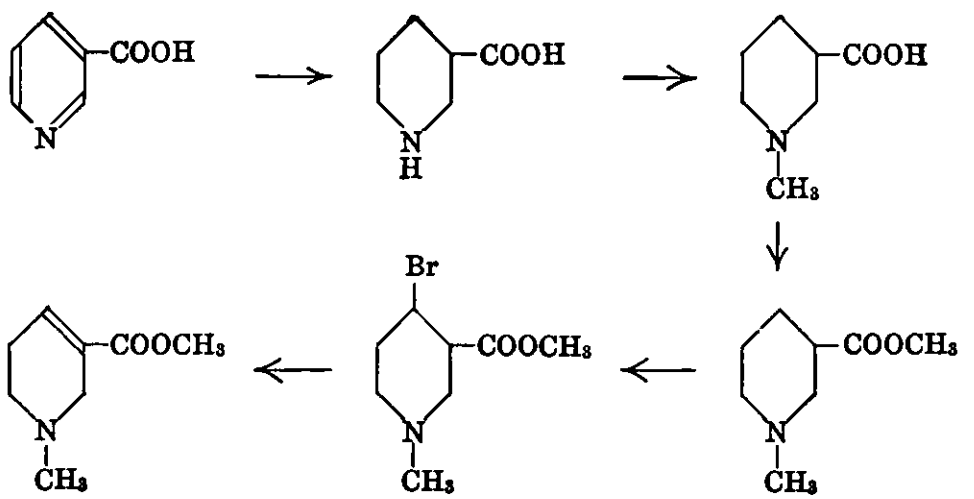
material present. Extraction of the carbon tetrachloride liquor with 10% ammonia is repeated once more. All the clear, aqueous ammoniacal solutions are then combined, are carefully acidified by the addition of sulfuric acid, and are concentrated under vacuum to a small volume. The arecaidine can now be precipitated by the addition of potassium bismuth iodide, the compound can be filtered off, and the alkaloid liberated as previously described (see diagram). It is transformed into the methyl ester in the usual manner with methanol and dry hydrochloric acid gas.

SYNTHESIS OF ARECOLINE

Of the several syntheses of arecoline which have been published in the literature, all but one have been adapted to large-scale processing. The manufacture of arecaidine from nicotinic acid has been found to be rather tedious and unreliable, with varying and very often low yields. Of the other methods, all give rather good yields, and the choice depends mainly upon the type of equipment available. For that reason, all possible methods will be described here, but the reader is referred to the original literature for details.

From Nicotinic Acid

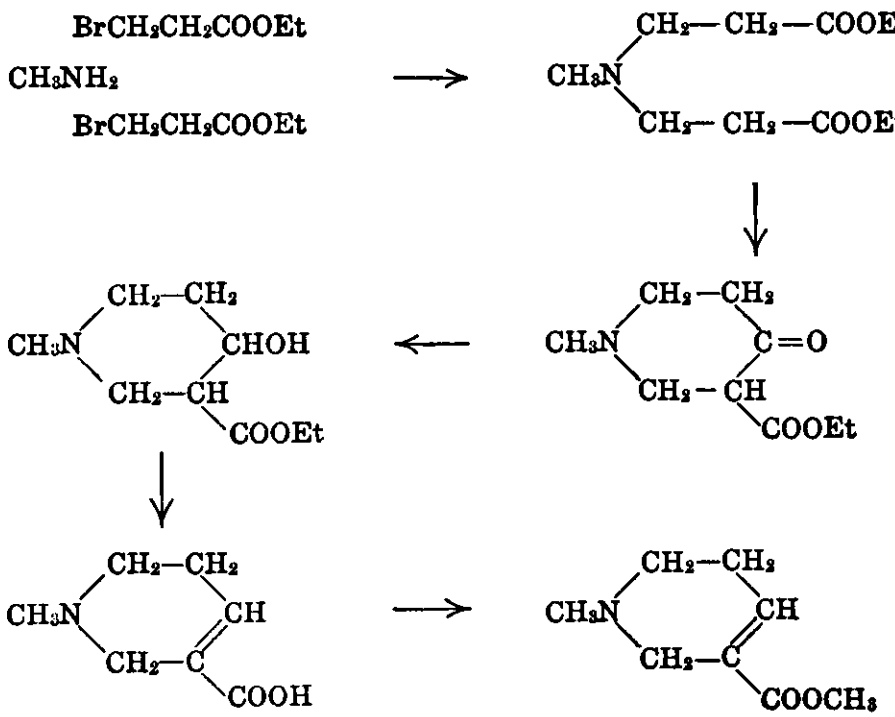
Nicotinic acid can easily be hydrogenated to hexahydronicotinic acid,[8] which, on N-methylation, esterification, bromination in the 4-position followed by debromination yields arecoline.[9] Preobrazhenskii and Fisher[10] have voiced some doubt concerning the last step. As mentioned before, this method is not too reliable.



From Beta-halogen Propionic Ester

This process is adapted from methods described by McElvain[11] and by Daukara *et al.*[12] Two pounds of dry gaseous methylamine is dis-

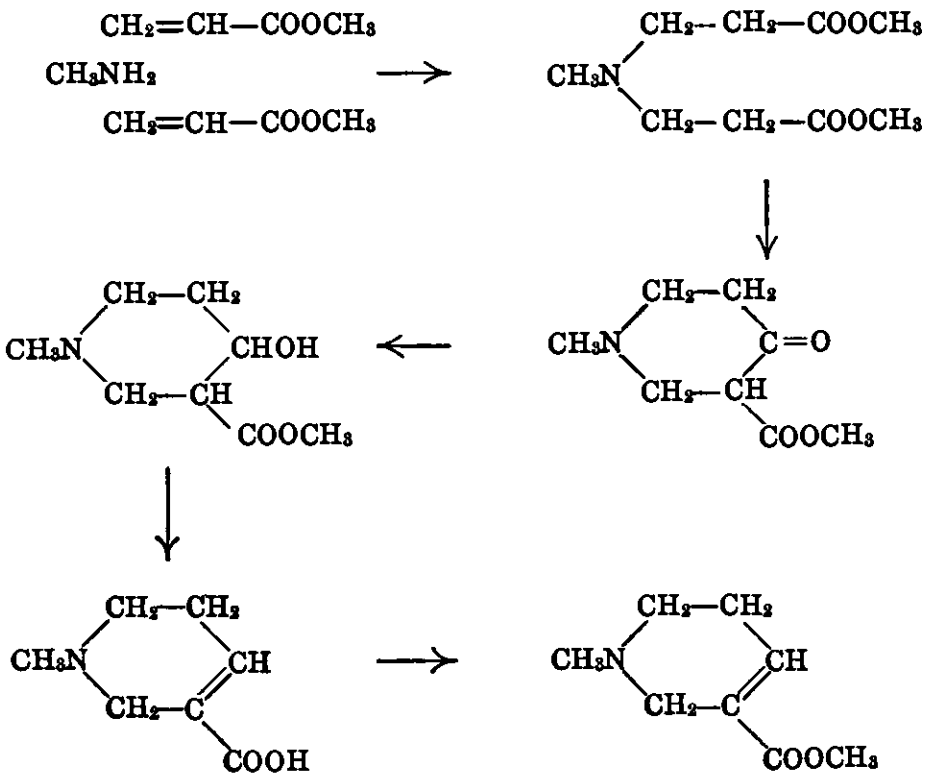
solved in 5 gal of dry benzol or toluol under cooling with ice; 10 lb of ethyl-beta-bromo-propionate is then added to this solution, and the mixture is heated in an autoclave at 150° for 3 hr. The excess of methylamine is necessary to neutralize the hydrobromic acid formed during the reaction. After cooling, the reaction product is washed twice with dilute potassium hydroxide solution, twice with water, and is distilled under vacuum after drying with anhydrous sodium sulfate. The residue, consisting of methyl bis-(2-carbethoxyethylamine) is distilled under vacuum (b.p.: 136°/4 mm Hg), yield 5.5 lb (90%). Ring closure (see diagram) to form 1-methyl-3-carbethoxy-4-piperidone can be effected in three different ways: by refluxing with sodium in isoamylalcohol (30% yield), by heating with sodamide (15–20% yield) in toluol, or by warming with sodium sand (10%) in xylene (70% yield). In the last two reactions care must be exercised, since the reaction usually starts suddenly at about 70° and proceeds exothermically from that point on. Instantaneous cooling of the reaction vessel, a wide and efficient reflux condenser, and a high-speed stirrer are essential. After the initial reaction subsides, refluxing should continue for a short period. Excess sodium or sodamide are then destroyed by the careful addition of methanol under cooling. The reaction product is then poured onto ice, extracted twice with ice-cold dilute hydrochloric acid. The base is then liberated by adding potassium carbonate, taken up by extracting exhaustively with ether, and is finally distilled (after drying) under vacuum (b.p.: 116°/4 mm Hg) (hydrochloride m.p.: 129°) (yield: 3.75 lb, 70%).



Reduction to 1-methyl-3-carbethoxy-4-hydroxy-piperidine can be effected with sodium amalgam in dilute hydrochloric acid, with aluminum isopropylate according to Meerwein-Ponndorf or reduction of the hydrochloride in absolute alcohol with Adams' catalyst. The base can be purified as usual, b.p.: 125°/4 mm, yield 2.75 lb (75%). Dehydration to arecoline can easily be effected by warming with glacial acetic acid containing some hydrochloric acid, by warming with thionyl chloride, or by boiling with strong hydrochloric acid (25%) and potassium iodide. Arecaidine is thus obtained in a yield of 60–70%. It can easily be esterified to form arecoline.

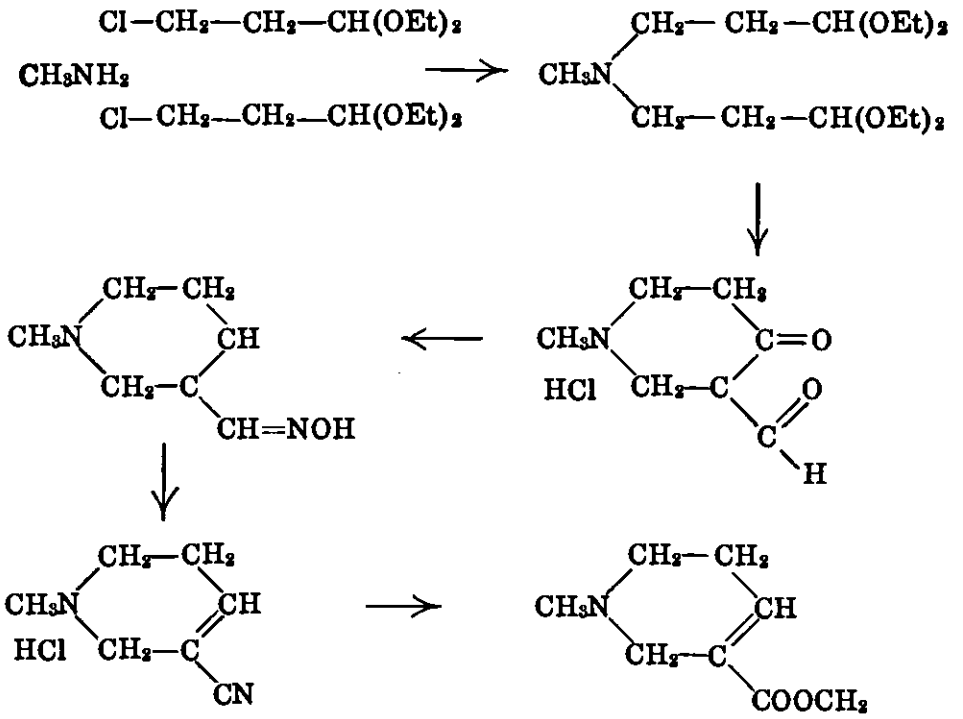
From Methylacrylate

Five pounds of methylamine (dry) are dissolved in 5 gal of dry methanol under cooling with ice. To this solution is now added 25 lb of methylacrylate under stirring. During this addition the temperature should be kept at about 25°. The reaction is brought to completion by warming for a short time. The methanol is then removed under vacuum and the diester is also distilled under vacuum (b.p.: 105°/4 mm) (yield: 15 lb (75%)). From here on, the process is identical to the one described in the preceding section, only the physical constants being different as follows: 1-methyl-3-carbomethoxy-4-piperidone, b.p.: 94°/4 mm; 1-methyl-3-carbomethoxy-4-hydroxy-piperidine, b.p.: 99°/4 mm. Yields are identical with the ones in the process for beta-halogen propionic ester:



From Propionaldehyde

Wohl and Johnson [13] described the condensation of beta-chloropropionaldehyde acetal with methylamine (b.p.: 127°/4 mm), followed by ring closure with fuming hydrochloric acid to form the hydrochloride of the aldehyde (m.p.: 195°), formation of the oxime (m.p.: 250°), dehydration to the unsaturated nitrile with thionyl chloride (hydrochloride, m.p.: 230°), and finally hydrolysis and esterification to form arecoline.



From Acetaldehyde

This method was originally published by Mannich.[14] This process is similar to the one above, which starts from chlorpropionaldehyde, but it has the advantage that the raw materials are more easily available.

One pound of dry methylamine hydrochloride is dissolved in an equal weight of water, and to the mixture is added 40 oz of aqueous formaldehyde (38–40%) and 40 oz of acetaldehyde. The mixture is kept in an autoclave for 18 hr at about 70–80°. After cooling, the clear solution is evaporated under vacuum at a low temperature to one half of its volume. At this point, a methanolic solution of hydroxylamine is added, which has been prepared by treating a solution of 11 oz of hydroxylamine hydrochloride in methanol with the calculated amount of sodium methoxide dissolved in methanol and filtering out of the sodium chloride. The solution of the oxime is now made strongly acid by the addition of alcoholic hydrogen chloride and is then distilled to dryness. The oxime hydrochloride can be recrystallized from isopropanol or from absolute

ethanol (m.p.: 250°) (yield 8 oz). This oxime is then converted into the nitrile by warming for 1 hr on the steambath with five times the amount of acetic anhydride followed by boiling for a very short time, evaporation to dryness and recrystallization from 95% ethanol (yield 7 oz. m.p.: 228°). This nitrile hydrochloride can be hydrolyzed with methanolic hydrogen chloride to form arecoline.

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

Of the Areca alkaloids, arecoline is the only one possessing toxic properties and some pharmaceutical value. Because of its toxicity in larger doses which may bring about central paralysis, its use in human medicine has been abandoned. It is now used exclusively in veterinary medicine as a vermifuge.

Because of its parasympathetic stimulant action, arecoline seems to belong, pharmacologically speaking, into the muscarine pilocarpine group.[15] In this respect arecoline acts more powerfully than pilocarpine. Other actions resembling those of pilocarpine consist of contraction of the pupil of the eye, stimulation of the secretion of the salivary glands, constriction of the bronchii.[16] Arecoline reduces intraocular tension, but its effects are of shorter duration than that of pilocarpine or physostigmine, and its use in ophthalmology has been all but discontinued.

Arecoline induces intestinal peristalsis, and it is poisonous to the roundworm but seemingly without effect on the hookworm.[17] Its main use now is because of these effects, especially in the treatment of horses for wind colic or as a cathartic.[18] Some European *Pharmacopœiae* recognize it because of its sialogogic and diaphoretic actions. Arecoline can be recovered unchanged from the secretions and excretions of the body.

ANALYTICAL METHODS

Arecaidine, in solution in dilute mineral acid, gives precipitates with bismuth potassium iodide, Mayer's reagent, with potassium iodide (in acid solution only, not in neutral solution). Picric acid gives no precipitate, but phosphomolybdic and tannic acids show a turbidity.

Arecoline gives a greenish-white precipitate with Mayer's reagent, a pomegranate-red microcrystalline precipitate with bismuth potassium iodide, and a white precipitate with phosphomolybdic acid. Picric acid yields a resinous precipitate which turns crystalline on standing. Tannic acid gives no precipitate.

Precipitates are also formed at once with trinitrophenolphthalein, tetranitrophenolphthalein and hexanitrodiphenylamine. Trinitro-

phloroglucine gives a crystalline sediment after several hours. Arecoline gives a blue color with potassium ferricyanide and a green color with potassium ferrocyanide.

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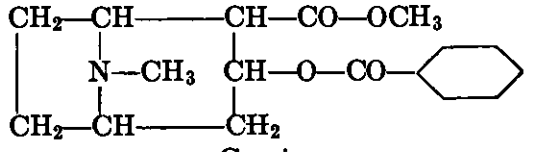
CHAPTER V

THE ALKALOIDS OF COCA

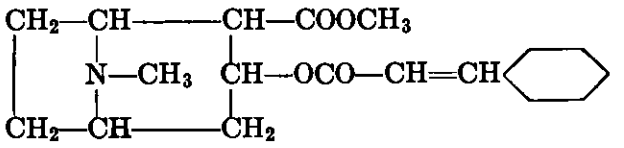
CHEMISTRY

The alkaloids of coca can be classified into four groups: the true cocaines, the pseudotropeines, the acylecgonines, and the hygrines. Only the alkaloids of the first and the third group, those which contain ecgonine or are derivatives thereof, are of commercial importance.

The most important alkaloids of the first group are cocaine itself and cinnamyl-cocaine, but alpha- and beta-truxilline are also present in small amounts.

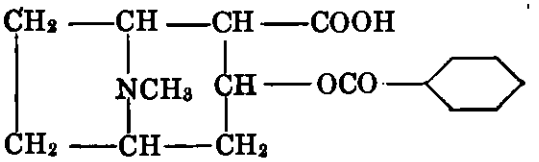


Cocaine



Cinnamyl-cocaine

Benzoylecgonine is the most important representative of the third group mentioned above. The secondary alkaloids of coca will be mentioned shortly at a later place.



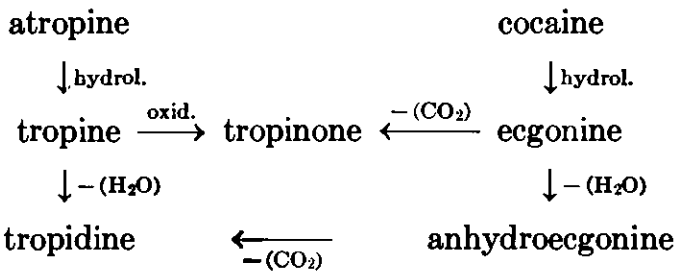
Benzoylecgonine

It has been indicated previously that ecgonine is common to all these alkaloids. The laevo-form of that base is obtained from all these alkaloids by hydrolysis with acid,[1] while the dextrorotatory isomer (*d*-pseudoecgonine) is obtained by alkaline hydrolysis.[2] Racemic pseudoecgonine has been obtained by synthesis.[3]

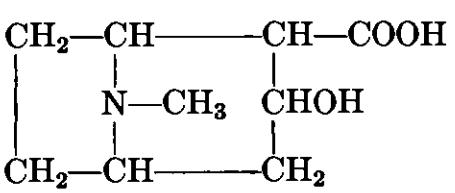
Laevo-ecgonine, $C_9H_{15}O_3N \cdot (H_2O)$, obtained from the natural alkaloids, was found to contain a hydroxyl as well as a carboxyl group, and to furnish salts with acids as well as with bases. With dehydrating agents the carboxyl group is left intact, but a double bond is formed and anhydroecgonine, $C_9H_{13}O_2N$, is formed.[4] Decarboxylation takes place by heating with hydrochloric acid to 280°, and tropidine is obtained from anhydroecgonine.[5]

The close relationship of ecgonine and tropine can also be seen from the following degradation products of ecgonine: Oxidation with chromic acid yields tropinone, tropinic acid, and ecgoninic acid,[6] and the structure of the last-named compound has been confirmed by synthesis.[7]

The following diagram will serve to illustrate further the relationship between cocaine and atropine



Also similar to the reaction observed by the degradation of tropine, ecgonine can be oxidized to nor-ecgonine, $C_8H_{13}O_3N$, which is a secondary base, the N-methyl group having been oxidized.[8] The Hofmann degradation of anhydroecgonine has also been examined.[9] In all the analogous degradations of tropine and ecgonine it was found that the latter differed from the former by CO_2, suggesting the presence of a carboxyl group in ecgonine. Several formulae have been suggested from ecgonine,[10] but the following formula was finally adopted:



Some doubt concerning the relative positions of the hydroxyl and carboxyl groups was resolved by a consideration of the following reactions: The mechanism of the oxidation of ecgonine to tropinone,[11] and the products of the reduction and further degradation of anhydroecgonine ethyl ester.

Pseudoecgonine has been prepared from tropinone by carboxylation with carbon dioxide [12] as well as by other methods.[13] Reduction of tropinone sodium carboxylate obtained above with sodium and alcohol

gave some *d,l*-pseudoecgonine, but the reduction of tropinone methyl carboxylate [14] gave a mixture of *d,l*-pseudoecgonine and *d,l*-ecgonine. The former could be resolved in the form of its methyl ester and the corresponding optically active pseudococaines were synthesized. The racemic ecgonine was first converted into racemic cocaine which could later be resolved into its optically active antipodes. The relation of ecgonine to pseudoecgonine is thought to be similar to that between tropine and pseudotropine.[15]

Cocaine, $C_{17}H_{21}O_4N$, was found to be methyl-benzoyl-*l*-ecgonine by the following series of reactions and degradations: when cocaine is boiled with water, it decomposes into methanol and benzoyl-*l*-ecgonine. The latter can be hydrolyzed further into *l*-ecgonine and benzoic acid, or cocaine, on acid hydrolysis, furnishes methanol, benzoic acid, and *l*-ecgonine directly.

Cocaine can be crystallized from alcohol. It is quite soluble in that solvent as well as in petroleum ether, benzol, ether, and other hydrocarbon solvents, but only slightly soluble in water, where it shows an alkaline reaction. The salts are crystalline, and besides the hydrochloride salts with organic acids have been used in medicine.

d-Pseudococaine [16] probably does not occur as such in the coca leaves, but is formed during the extraction and purification of cocaine by isomerization under the influence of alkali. Its stereochemical relationship to cocaine has been proved by its synthesis [17] from *d*-pseudoecgonine. The salts are less soluble than the corresponding ones of cocaine.

Racemic pseudococaine has been synthesized by Willstaetter *et al.*[18]

Cinnamyl-cocaine, $C_{19}H_{23}O_4N$, has been prepared from Java coca,[19] and it has also been synthesized.[20] It can be hydrolyzed to cinnamic acid, methanol, and *l*-ecgonine. The alkaloid can be crystallized from benzol or petroleum ether. It is soluble in most organic solvents but almost insoluble in water. Its salts are crystalline. Its optical isomer, *d*-cinnamyl-cocaine, has been prepared from *d*-pseudoecgonine methyl ester and cinnamoyl chloride.[21]

Benzoylecgonine, $C_9H_{14}O_2N(OCOC_6H_5)$, has been prepared from the natural drug as well as synthetically.[22] The alkaloid can be hydrolyzed to *l*-ecgonine and benzoic acid, or it can be esterified with methanol to cocaine. Homologues of cocaine have been prepared by esterification with alcohols other than methanol.

Methylcocaine (ethyl-benzoyl-*l*-ecgonine?) has been prepared by pseudosynthesis from cocaine by fractional precipitation of its solution in ethanolic hydrochloric acid.[23] The base is isomeric, but not identical, with a compound obtained by the esterification of benzoylecgonine with ethanol.[24]

Alpha and beta truxilline, $C_{38}H_{46}O_8N_2$, have been prepared in a pure state by synthesis.[25] The separation of the mixed alkaloids obtained from natural sources [26] has proved difficult.[27] Acid hydrolysis of the two isomeric alkaloids gave rise to *l*-ecgonine, methanol and alpha- and beta-truxillic acids respectively. The former was shown to be gamma-iso-atropic acid, whereas the latter is delta-iso-atropic acid.

The pseudotropeines and hygrines, although of little commercial importance, are of some scientific interest. Tropacocaine, $C_{15}H_{19}O_2N$, has been found in Peruvian and in Java coca leaves.[28] It can be hydrolyzed to benzoic acid and pseudotropine and it is made commercially by benzoylation of pseudotropine, obtained from tropine by treatment with sodium amylate in amyl-alcohol. Tropine and pseudotropine are thought to be cis-trans isomers.[29] From the group of hygrines, at least three alkaloids have been isolated, hygrine, $C_8H_{15}ON$,[30] beta-hygrine, $C_{14}H_{24}ON_2$,[31] cuscohygrine, $C_{13}H_{24}ON_2$.[32]

NATURAL SOURCES

Coca leaves are no longer official in the United States or in Great Britain. They were last mentioned in the *U. S. Pharmacopœia* (8th Edition) and in the *British Pharmacopœia* of 1898. They were then described as "the dried leaves of *Erythroxylon coca* Lamarck, known commercially as Huanuco Coca, or the leaves of *Erythroxylon truxillense* Rusby, known commercially as Truxillo Coca, or the leaves of *Erythroxylon novogranatense* (Morris) Hieron, known commercially as Peruvian Coca."

Erythroxylon coca and *truxillense* are thought to have occurred originally in Peru and Bolivia only, but they are now found in many parts of South America. The latter variety is thought to be synonymous with *Erythroxylon novogranatense*, originally found in Colombia.[33]

The plants are cultivated in Peru and Bolivia on the mountain slopes, 1500–6000 ft above sea level, preferably in moist climates. The shrubs attain a height of about 6 ft. They have slender branches, alternate, oval, entire and elliptic leaves, small whitish or yellowish flowers, and red, one-seeded fruits. The seeds are first grown in nurseries. The young plants are transplanted to terraces which are built on the slopes of the mountain in a step-like arrangement. Leaves can be harvested after 18 months, but the plant attains its best yield after 3–5 years, when three to four harvests are obtained annually. The leaves are collected, and are first dried in closed chambers and then on open pavements. Peruvian or truxillo leaves can be distinguished from the Huanuco or Bolivian coca by being somewhat smaller, thinner and more

membranous, with the lateral lines on the lower side being absent, obscure, or incomplete. The Bolivian variety shows two distinct lines on either side of the midrib. The Peruvian leaves are pale green, brittle, and usually considerably broken. The Huanuca leaves are of brownish-green color and mostly entire. They are up to 8 cm long and up to 4 cm wide. The plants are also cultivated extensively in Java, Ceylon, and Formosa. Full descriptions of all varieties can be found in the *U. S. Dispensatory.*

The following average amounts of alkaloids have been found in different drugs:

Origin	*Total Alkaloids, %* (*Average*)
Peru	0.3–1
Bolivia	0.7–1
Java	0.6–2.5
Ceylon	0.6–1.6

MANUFACTURING METHODS

Although considerable quantities of crude cocaine are imported to serve as a source of pure cocaine, that alkaloid is also produced in this country by extraction from the coca leaves. The manufacture of cocaine is usually performed on a large scale, often protected by patents, and only a survey of the possible methods is offered here.

The powdered coca leaves can be conveniently extracted with dilute (3%) sulfuric acid, until a sample shows only a slight opalescence with Mayer's reagent. Three extractions are sufficient as a rule. The extracts are combined; the alkaloids are precipitated with sodium carbonate or lime and are dissolved in a nonhydroxylic solvent, for instance, toluol or kerosene. Any emulsions which might form at this stage are broken by filtration with charcoal and filteraid. Two extractions are often necessary to remove all the alkaloids from the alkaline solution.

The solvent solutions of the alkaloids are combined and the alkaloids removed by extraction with 10% sulfuric acid. In this solution, the total alkaloids are determined approximately and calculated as cocaine. For each 100 lb of cocaine, approximately 30 lb of free (or excess) sulfuric acid should be present. This acid solution is now boiled for about 12–24 hr to hydrolyze all the alkaloids present. The solution is then cooled, filtered, and extracted with toluol or kerosene to remove organic acids. The aqueous phase is then treated with potassium carbonate until it is only slightly acid to litmus test paper. After filtration it is concentrated under vacuum to a thick syrup, which is then diluted with 10–15 times its volume of alcohol. The inorganic material is filtered off, and the

filtercake is washed with alcohol. The filtrate and washings are combined and concentrated, and the *l*-ecgonine sulfate is allowed to crystallize at a low temperature. The motherliquors from this material contain some unsaponified material, which is returned to the process for further saponification. Cocaine is then resynthesized from the *l*-ecgonine.

According to a different method, the powdered leaves are intimately mixed with a dilute solution of sodium carbonate or lime, and the total alkaloids are then extracted with kerosene. The alkaloids are removed from the kerosene by extraction with dilute hydrochloric acid, and they are finally obtained either by precipitation with ammonia, or by concentration of the acid solution and crystallization of the hydrochlorides. On the other hand, *l*-ecgonine can be obtained by somewhat concentrating the original hydrochloric acid solution, boiling it for several hours, removing organic acids by filtration or extraction with kerosene or toluol, concentrating the motherliquors, and crystallizing the *l*-ecgonine from alcohol and ether. The crude, precipitated total alkaloids have also been hydrolyzed in methanol in the presence of sulfuric or hydrochloric acid, whereby the methyl ester of ecgonine is obtained directly. Ten pounds of crude total alkaloids are boiled with 100 gal of methanol and 22–25 lb of concentrated sulfuric acid. The methanol is distilled, the residue is dissolved in water, organic acids are extracted with ether or toluol, the aqueous phase is made alkaline by adding, under cooling, an excess of potassium carbonate, and the *l*-methylecgonine is extracted with chloroform or ether.

The following method of purifying crude cocaine has been suggested by de Rosemont: [33a] The crude alkaloid is dissolved in hot water, which contains half its weight of naphthalene-beta-sulfonic acid. The solution is filtered and allowed to cool somewhat, and it is then decanted from the precipitate which forms. From the clear solution, the pure cocaine is precipitated with ammonia. The naphthalene sulfonic acid is precipitated from the motherliquors with hydrochloric acid after concentration. The residues from which the liquid had been decanted can be hydrolyzed to obtain further amounts of *l*-ecgonine.

Pure cocaine is then resynthesized from *l*-ecgonine by several methods, some of which have been protected by patents.[34] The procedure is similar in most cases. *l*-Ecgonine is first esterified with methanol and then with benzoic acid. Esterification with methanol is undertaken as described previously, dissolved in an excess of methanol and under the influence of hydrochloric acid gas or of concentrated sulfuric acid. The latter sometimes leads to the formation of some anhydroecgonine.

Several methods have been used successfully in esterifying the

l-methylecgonine with benzoic acid. The former can be heated with a slight excess of benzoic anhydride or benzoyl chloride for several hours to 150°, or it can be heated with benzoyl chloride to 120° until no more hydrochloric acid gas is evolved; the resulting solution can be cooled to 100° and then poured into dilute aqueous ammonia to precipitate the cocaine; it can also be heated with benzoic acid and phosphorous oxychloride, or it can be boiled with benzoic anhydride in benzene or toluene, thus removing the water which forms by continuous distillation. When phosphorous oxychloride is used, 10 parts of methylecgonine are heated with 8 parts of benzoic acid to 120°, and 3 parts of phosphorous oxychloride are added slowly.

The crude cocaine, which is obtained from the reaction mixtures by careful dilution with aqueous hydrochloric acid, is precipitated from that solution with ammonia. It is almost always recrystallized from a mixture of acetone and benzol.

The crude cocaine is often purified in the following manner: The crude base is dissolved in 5% sulfuric acid, the solution is cooled to 0°, and a cold, concentrated solution of potassium permanganate is added slowly under stirring, until a slight coloration remains unchanged for about 5 min. The alkaloid is then precipitated, and the hydrochloride is crystallized from alcohol and ether after treating with charcoal.

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

Cocaine has two distinct forms of action, one local and the other systemic. It is mainly because of the latter one that the use of cocaine is strictly regulated.

When cocaine is applied locally it paralyzes the peripheral ends of the sensory nerves and, with lesser intensity, those of the motor nerves. The muscular coats of the blood vessels are slightly stimulated. When applied to oral or nasal membranes, the senses of taste and smell are also diminished.

Internally, the whole nervous system is stimulated, including the medulla, the brain, and the spinal cord. Administration of cocaine is followed by exaltation, which may be followed by delirium in the case of larger doses, increasing reflex activity and increased rapidity of respiration, which may be followed by a depression of the respiratory center. The blood pressure may also be increased,[35] but its cardiac effect is doubtful.[36] When introduced into the eye, cocaine causes dilatation of the pupil.

In medicine, cocaine is used both as a systemic and as a local remedy, but because of its habit-forming property its use as a systemic drug is

not always advisable. Its secondary action as a depressant also somewhat restricts its use.

ANALYTICAL METHODS

Solutions of cocaine yield precipitates with alkali hydroxide and carbonates and with ammonia, Mayer's reagent, phosphomolybdic acid, tannic acid, platinic and auric chloride, and potassium permanganate. All these reagents, except perhaps the tannic acid and the potassium permanganate, give precipitates even in very dilute solutions. The precipitates obtained with aurichloride and with platinic chloride are rather characteristic, and can be used for the microchemical identification of cocaine.[37]

In cases of cocaine poisoning, the alkaloid can be found in the digestive and urinary tracts, the liver, or other organs, depending upon the mode of administration.

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33a. *J. Suisse de Pharm.*, April 29, 1929.
34. German Patents 47, 602; 298, 678; 301, 498. *Ber.* **27,** 1523 (1894); **18,** 2953 (1885); **21,** 3196, 3335 (1888). *Chem. Ztg.* **54,** 31 (1930); *J. Soc. Chem. Ind.* **8,** 724, 1013 (1889). *J. Prakt. Chem. II* **116,** 276 (1927). *Chemist and Druggist,* 1920, 934; 1912, 80, 51.
35. Crosby, *J. Pharm. Exp. Therap.* **65,** 150 (1939).
36. Reichert, *Penn. Med. J.*, 1902. Kuroda, *J. Pharm. Exp. Therap.* **7,** 423 (1915). Prus, *Zt. Exp. Path. & Therap.* **14,** 161 (1913).
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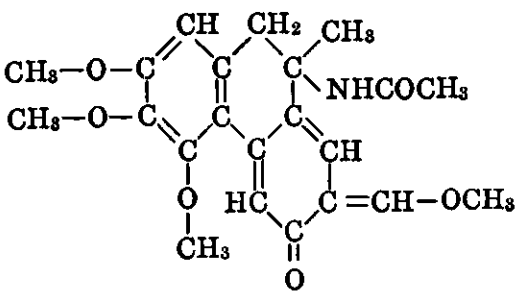
CHAPTER VI

COLCHICINE

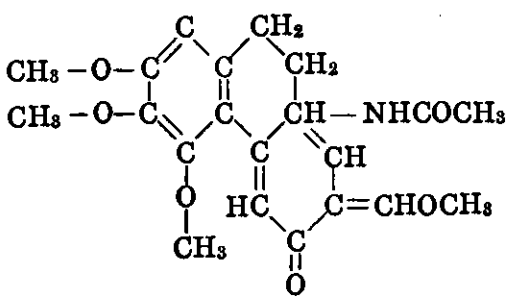
CHEMISTRY

Colchicine is the only alkaloid occurring in *Colchicum autumnale* Linné, where it is found in all parts of the plant, but mainly in the seeds and in the corm. Colchiceine has been found to accompany it in the plant, but it is thought not to occur as such but to be a product of the hydrolysis of colchicine.[1]

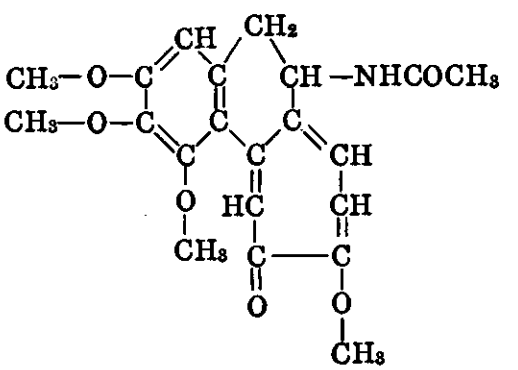
The structural formula of colchicine, $C_{22}H_{25}O_6N$, is not absolutely certain yet. The classical formula is that of Windaus,[2] who thought it to be a phenanthrene derivative, somewhat resembling the morphine alkaloids.



Windans (1916)



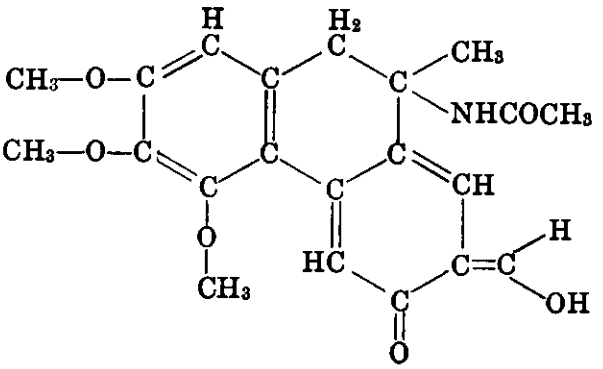
Tarbell (1946)



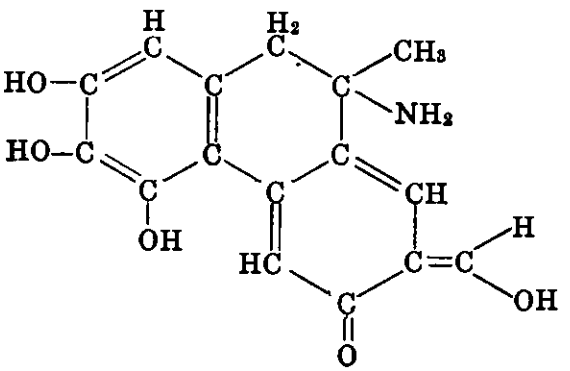
Dewar (1945)

It was found that colchicine contains one rather unstable methoxy group, three other methoxy groups which proved to be more resistant against hydrolysis, and one acetyl group. When the latter was removed, the remaining base behaved like a primary amine.

Colchicine loses one mole of methanol under the influence of hot, dilute hydrochloric acid to form colchiceine. When stronger acid is used, one mole of acetic acid is also split off and trimethylcolchicinic acid is formed, which is amphoteric in character, and which can be reconverted into colchicine by methylation and acetylation. Upon demethylation three methoxy groups are removed, and colchicinic acid results.

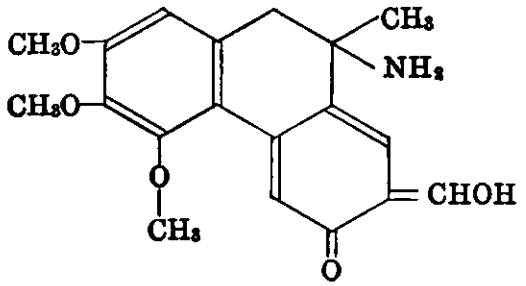


Colchiceine
(Windaus)

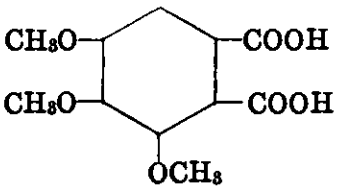


Colchicinic acid
(Windaus)

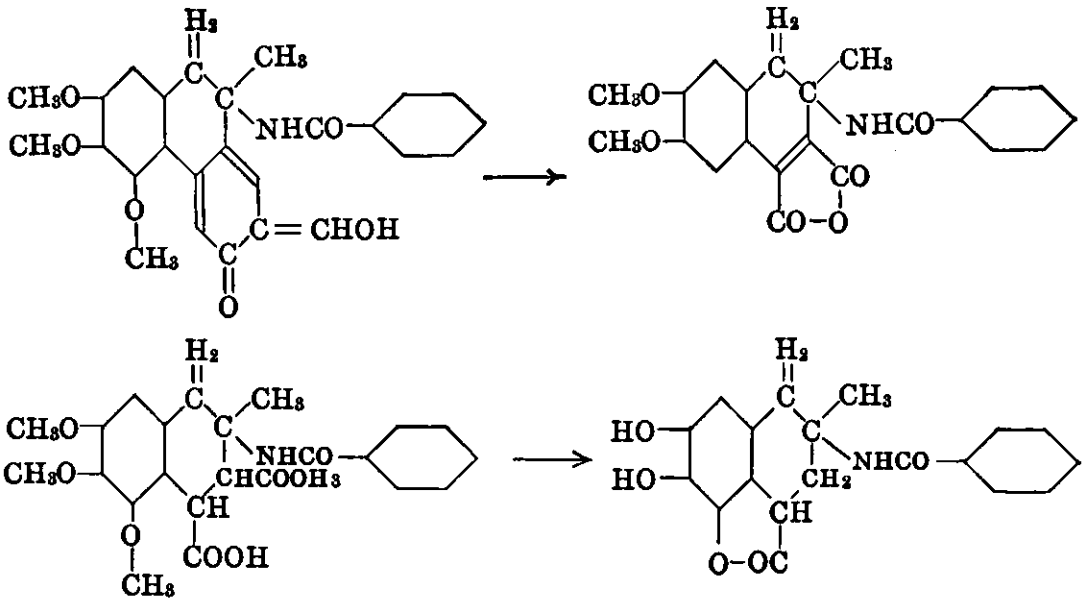
Oxidation of colchicine, colchiceine, or trimethylcolchicinic acid with hot permanganate yields the same product in each case, 3,4,5-trimethoxy phthalic acid, thus proving the presence of a second ring and the fact that the three methoxy groups are adjacent.



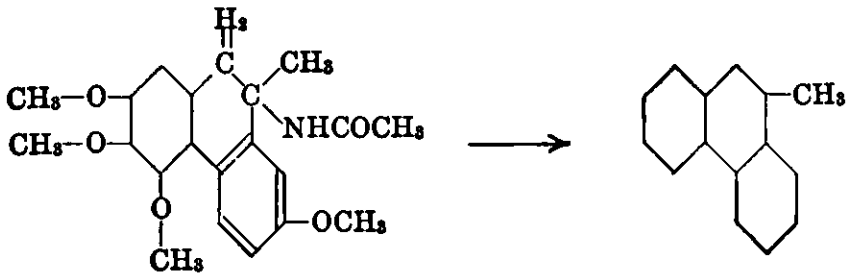
Trimethylcolchicinic acid



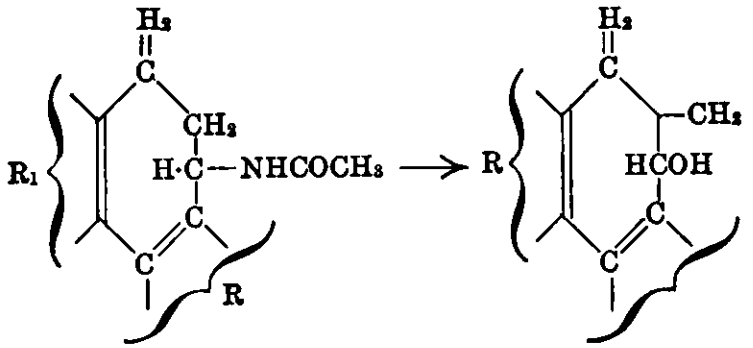
The position of the three methoxy groups in ring A was determined when N-benzoyltrimethyl colchicinic acid was subjected to an oxidation, followed by a reduction with zinc dust, whence a tetrahydronaphthalene derivative was obtained which could be transformed into a lactone. Since a lactone of that type can only be formed, according to Sachs,[3] when a hydroxy and a carboxy group are in the peri position, proof is given of the previous presence of a hydroxy group at position 4, which is derived from a methoxy group. The position of the three vicinal methoxy groups in relation to the second ring is therefore determined.



Windaus [4] assumed colchicine to be a phenanthrene derivative. He based this view on the following observation: Colchiceine on treatment with iodine and alkali is converted into N-acetyl-iodocolchinol, $C_{20}H_{22}NO_5I$, which could be transformed into N-acetyl-colchinolmethyl ether. After de-acetylation the free amine was transformed into the quaternary ammonium hydroxide. By heating under vacuum, trimethylamine was split off and a substance $C_{19}H_{20}O_4$ was obtained, which, after demethylation and distillation with zinc dust, gave 9-methylphenanthrene, which was further identified by independent synthesis.



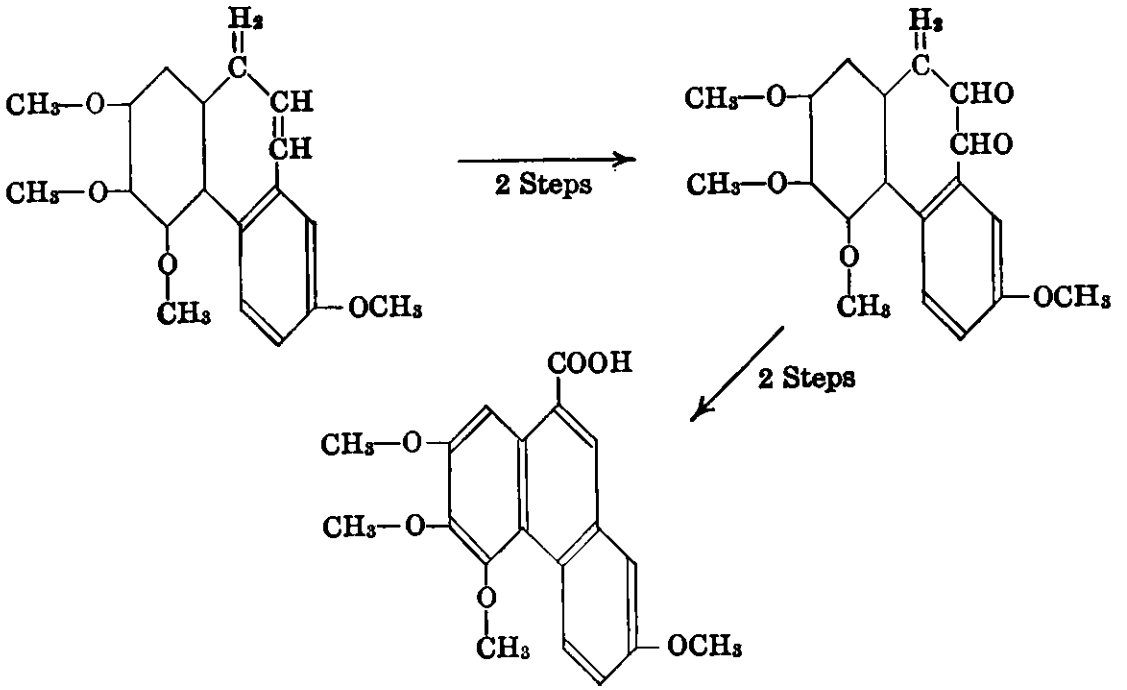
Colchinolmethyl ether, which can easily be obtained from N-acetoxy-iodocolchinol, if it conforms to the Windaus formula, should easily pass into the completely aromatic state by loss of ammonia. It was, however, pointed out by Cohen *et al.*[5] that no evidence of such instability exists. They obtained a carbinol by diazotation of colchinolmethyl ether, which did not react with phthalic anhydride in benzol, but which gave an acid phthalate upon fusion with phthalic anhydride. This series of reactions is more characteristic of a secondary alcohol, but does not exclude a primary alcohol. Considering the possibilities of a drastic intramolecular change in the course of a distillation with zinc dust (formation of 9-methylphenanthrene) more likely than a rearrangement during diazotation, Cohen *et al.*[5] advanced the possibility of a seven-membered ring B. The presence of at least one methylene group in ring B had previously been proved by the conversion of colchicine into oxycolchicine by oxidation with chromic acid, analogous with the formation of benzophenone from diphenylmethane.



Proof of the constitution of ring B was recently given by Tarbell *et al.*[6] They examined deaminocolchinolmethyl ether, which, according to Windaus, should be either 2,3,4,6- or 2,3,4,7-tetramethoxy-9-methylphenanthrene. This was found to be inconsistent, since the compound did not form a picrate and could be reduced with palladium to a dihydride, while synthetically prepared tetramethoxymethylphenanthrene derivatives could not be reduced under similar conditions.

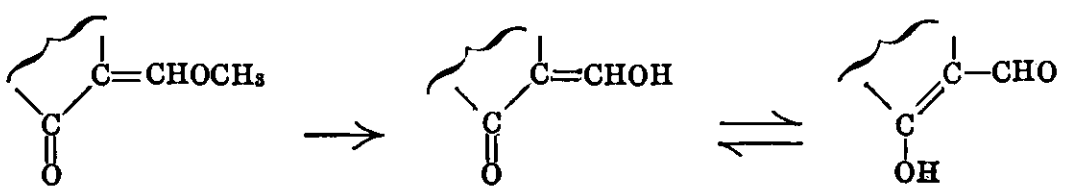
Deaminocolchinolmethyl ether was oxidized with selenium tetroxide and the diol so obtained was cleaved with lead tetra-acetate. The

dialdehyde so obtained, after undergoing aldol condensation, was oxidized to an acid, the structure of which was proved by synthesis.



Deamino-iodocolchinolmethyl ether, on oxidation with potassium permanganate in acetone at room temperature, gave a dicarboxylic acid, which was esterified with diazomethane and treated with sodium methylate to yield a phenanthrol derivative.

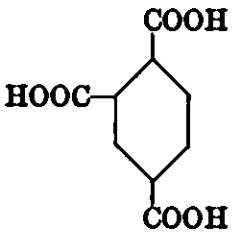
Priority for the idea of a seven-membered ring B belongs to Dewar,[7] who drew the analogy to stipitatic acid and suggested calling the seven-membered ring "tropolone ring." He pointed out that his formula could account for many hitherto unexplained facts: namely, that colchiceine is a much stronger acid than salicylaldehyde, that it gives no carbonyl or aldehyde reaction, and that the adsorption spectra of colchicine and colchiceine are almost identical (Bursian, *loc. cit.*); it also explains the ferric chloride reaction of colchiceine, and the existence of two isomeric dibenzenesulfonyl derivatives (Windaus).[8]



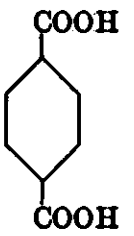
Colchicine **Colchiceine** **Enol**

The presence of a third ring in colchicine was shown when its deaminated product obtained by caustic fusion was oxidized with permanga-

nate to yield terephthalic and trimellitic acids:

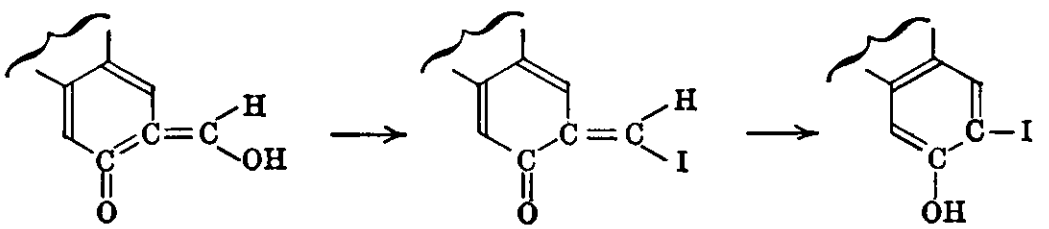


Trimellitic acid

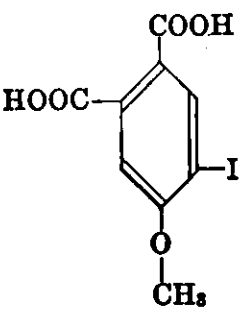


Terephthalic acid

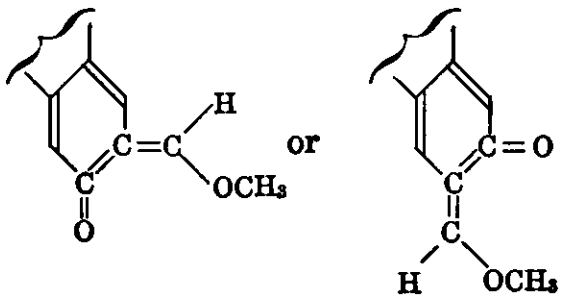
Windaus [9] showed that colchiceine behaved as a keto-enolic or as an aldehydic substance and thought it to be a beta-keto-aldehyde. Oxidation with bromine in glacial acetic acid gives rise to tribromocolchiceinic acid, the carbonyl group of which can only have originated in an aldehyde group. Iodine in the presence of alkali converts colchiceine into N-acetyl-iodocolchinol, probably by the replacement of an aldehyde group by iodine.



It has been shown by Grewe [10] that the iodine and methoxy groups must occupy adjacent positions, at carbons six and seven, because this ether on oxidation with nitric acid gives rise to 5-iodo-4-methoxy phthalic acid, whose identity was established by synthesis.

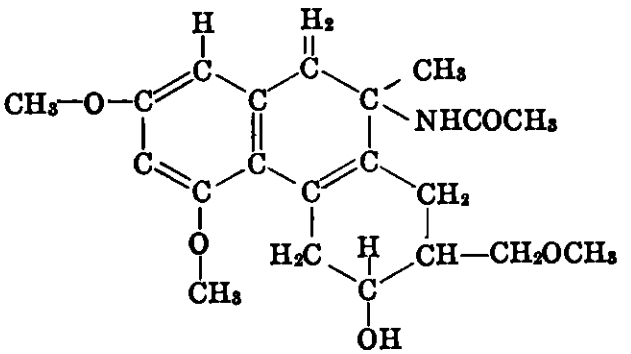


The hydroxy-aldehyde formula was slightly modified, taking into account the failure of colchicine to give aldehydic reactions, and the labile nature of the methyl-ether group in colchicine. Either of the following formulae can then be considered to represent colchicine, although Dewar's formula is also quite possible.



Colchicine
(Bursian 1938)

Colchicine on hydrogenation in the presence of platinum (Adams and Shriner) was shown to adsorb three moles of hydrogen [11] forming a compound $C_{22}H_{31}O_6N$, which gives a benzoate and an acetyl-derivative, proving the conversion of a keto group into a secondary alcohol. It also shows the presence of a stable double bond besides the three double bonds in the benzenoid ring A. Because this double bond does not hydrogenate, and because titration with bromine yields a substitution product with the simultaneous liberation of hydrobromic acid, and because titration with perbenzoic acid yields a crystalline oxide, $C_{22}H_{31}O_7N$, this double bond is thought to be located between rings B and C.[11]



Colchicine, when sold in commerce, consists of yellow flakes or a yellowish powder (m.p.: 143–147°). It crystallizes from water as the trihydrate. Pure colchicine can be prepared by crystallizing the anhydrous powder from ethyl acetate from which it crystallizes in needles (m.p.: 155–157°). The specific rotation is a_D −121° in chloroform or a_D −429° in water. According to the *U. S. Pharmacopœia*, a gram of colchicine dissolves in 25 cc of water or in 220 cc of ether. It is quite soluble in dilute alcohol and in cold water, but is less soluble in absolute alcohol and in warm water, where only 12% is soluble at 82°. It is insoluble in petroleum ether and slightly soluble in benzol, but very soluble in chloroform from which it crystallizes at a low temperature in two different forms,[12] either with one or with two moles of chloroform

of crystallization. The chloroform is lost by drying at 100° in vacuo, and it is also split off in warm water.

Colchicine is very weakly basic and its salts are completely hydrolyzed in water. It forms a precipitate with tannic acid, which contains about 38% colchicine when dry. Colchicine forms an aurichloride (m.p.: 209°), soluble in alcohol, but does not give a precipitate with platinic chloride.

Colchiceine can be obtained by warming colchicine with 2% sulfuric or 1% hydrochloric acid. It crystallizes from water in colorless needles with ½ mole of water of crystallization (m.p.: 140-142°). The anhydrous product melts at 172–173°. It is insoluble in benzol and ether, slightly soluble in water, and soluble in alcohol and chloroform. Because of its amphoteric character it is soluble in both dilute alkalies and acids. Dilute mineral acids dissolve it with an intense yellow color. The solutions are laevorotatory.

NATURAL SOURCES

The meadow saffron, *Colchicum autumnale*, is a perennial herb, indigenous to Central and Southern Europe and North Africa, where it is found in rather moist places in pastures and meadows.

The ovoid corm grows several inches below the surface of the ground. One side of it is convex, while the other side is somewhat flattened and shows a groove. In the latter part of the summer a new corm begins to grow on the old one, growing half around the offshoots. From that base the new plant sends out its rootlets. In the late summer or autumn of the first year, two to six lilac- or purple-colored tubed flowers appear, but no leaves are present. The flowers, most of which are buried in the ground, disappear later in the autumn, and the fruit remains hidden in the ground until it is raised up during the following spring on top of a stem, forming a three-celled fruit. At the same time, three to five long, dark green leaves appear, and the new corm increases in size while the old one diminishes and finally disappears. The new corm, after having sent forth shoots and another new corm, disappears in its turn. In the second spring and before they disappear completely, old corms often generate one or more small corms which are the sources of new plants. New corms are formed each year on the lateral inferior surface of the old one.

One-year-old corms seem to have a maximum content of colchicine, and it has been recommended that the corms be collected before the flowering of the plant, which may vary from July to October according to the latitude. When the corm has been collected, the outer membranes

and rootlets are removed and the corm is sliced into several narrow sections and dried at a moderate temperature in an oven or in the sun.

The vitality of the corm and especially that of the growing spud is such that it may grow shoots even during the process of drying, unless it is deprived of its outer layer and sliced into sections. During the process of vegetation much of the colchicine is lost. The corm should therefore be dried quickly and carefully as soon as possible after removal from the ground. The best time for collecting the corm is from early June till the middle of August.

The seeds are found in the seed capsules in large amounts. They are small irregular spheres, or of an avoid shape, and they are somewhat beaked. Externally they are finely pitted and of dark brown color; internally they are of light grayish brown. They are collected in the late summer or early autumn, and they should also be dried carefully.

Cultivation of Colchicum has been attempted in this country but with not too much success. The usual method of cultivation consists in planting the corm in rows, 3–4 in. apart, and about 2–4 in. below the surface of the soil.[13]

Colchicum corms, as well as the seeds, are usually imported from Germany, Holland, Italy, or Yugoslavia.

The Colchicum seed, which is the crude drug most often employed for the manufacture of colchicine, is official in the *National Formulary* (8th Edition) and in the *British Pharmacopœia*. The first specifies a colchicine content of not less than 0.45%, whereas the latter requires a minimum of 0.3%. Foreign organic matter and acid insoluble ash are limited to 1% in the United States, but in Great Britain the corresponding figures are 2 and 3% respectively. The official description of the crude drug is essentially alike in the two countries, but the *British Pharmacopœia* also calls attention to the bitter acrid taste of the seeds.

The assay of Colchicum seeds described in the *British Pharmacopœia* is quite different from that described in the *National Formulary*. The latter requires extraction of the coarsely powdered seed with a warm aqueous solution of lead subacetate, which automatically removes a considerable amount of foreign inert matter, removing the excess lead from an aliquot portion of the filtrate, and extracting the clear filtrate with chloroform. The thoroughly dried residue remaining after evaporating the chloroform is weighed. The impurities are then removed with chloroform and dilute sulfuric acid, and weighed, and the difference between the two weights is colchicine.

The *British Pharmacopœia* prescribes extraction of the drug with 90% alcohol, dissolving the residue in water containing sodium sulfate, removing impurities by extraction with ether, making the solution alka-

line, and extracting the colchicine with chloroform and weighing the dried residue.

Colchicum corm is official in the *National Formulary* and in the *British Pharmacopœia*, the first prescribing a minimum content of anhydrous colchicine of 0.35%, the latter of 0.25%. Both books direct the assay to be carried out as described under Colchicum seed, but the *British Pharmacopœia* introduces an additional purification of the colchicine by dissolving it in water, thus separating it from the little soluble colchiceine and other inert matter.

The *French Pharmacopœia* also recognizes the flowers of Colchicum, a fluid extract of which is said to contain about 0.8% colchicine.[14]

The *British Pharmacopœia* recognizes Dry Extract of Colchicum, containing 0.9–1.1% colchicine, and a Colchicum Corm Fluid Extract (Addendum 7), containing 0.27–0.33% (weight per volume) of colchicine. The *National Formulary* recognizes Colchicum Corm Fluid Extract containing 0.3–0.4 grams of colchicine per 100 cc of extract, and Strong Colchicum Corm Tincture, containing 0.12–0.16 grams of colchicine per 100 cc of tincture.

Both Colchicum Seed Fluid Extract and Colchicum Seed Tincture are official in the *National Formulary* as well as in the *British Pharmacopœia*. The fluid extract contains 0.4–0.5 gram of colchicine, and the tincture 0.04–0.05 gram of colchicine per 100 cc, according to the *National Formulary*. The corresponding figures according to the *British Pharmacopœia* are 0.27–0.33% (weight per volume) and 0.027–0.033% of colchicine.

Colchicine itself is official in the *U. S. Pharmacopœia*, where its description and assay can be found.

MANUFACTURING METHODS

Either Colchicum corm or Colchicum seed can be used for the manufacture of colchicine, but the latter is to be preferred because of the considerably better yield.* It is desirable to grind to a mesh size of from 20–40 in the case of seeds; in the case of the corm the grind should be somewhat finer. The powdered drug is charged into the percolators and is then extracted as described previously. Seeds can be defatted with petroleum ether prior to extraction at this point.*

If a sufficient amount of drug is available, the countercurrent system of extraction is to be preferred. But if only smaller amounts of crude drug are at hand, the single batch system must be used. In that method the individual batches are charged into the different percolators and

* Private communication from Mr. R. L. Brown.

covered with solvent, which is then heated and circulated as described previously. The individual extractions are then drawn off and charged into the still for concentration.

The choice of the alcoholic solvent seemed to make little or no influence upon the final yield of colchicine. Denatured alcohol (95%), anhydrous methanol, and slightly diluted methanol (90–95%) have been used equally successfully, and experiments undertaken with isopropanol have shown some promise of success. It is important, however, to warm the alcohol to about 50°. As a rule, four extractions with warm alcohol are sufficient. A sample of the fourth extraction, upon evaporation in the laboratory, should give only a negligible residue.

Most of the solvent is distilled off under ordinary pressure, until the temperature of the batch in the still rises to about 80–85°. At that time vacuum is applied and more solvent is distilled off under vacuum until a fairly thick syrup remains in the still of about the consistency of a good heavy grade of honey. Depending on the amount of alcohol left in it, this residue will be about 5–15% of the original crude drug which it represents.

When the distillation is finished, about ten times the amount of water is added to the residue in the still and the material is allowed to cool under stirring for several hours. At that time, most of the residue from the distillation should have dissolved in the water. This mixture which contains some insoluble fats and resins can now be blown into the mixing tank as described previously. Although the batch itself will be cold, the blow pipes can be cleaned with steam.

To this mixture of colchicine solution and insoluble fats and resins in the mixing tank is now added 25–50 lb of filteraid for each 250 gal of liquid, representing about 15–50 lb of colchicine or 4000 lb of crude drug. Stirring is continued for ½ hr, and the batch is then filtered through a stoneware filter of adequate size (4-ft diameter). The filtered and clear liquor is returned to an empty mixing tank, and the filtercake is washed twice with cold water, using the equivalent of 10% of the total volume each time. The washings are combined with the main batch.

Defatting is now completed by washing the aqueous liquor twice with petroleum ether, using about 10% by volume of the liquor. The petroleum ether, after suitable stirring and settling (½ hr for each operation), is separated and held for recovery of the solvent. The residue from that recovery can be discarded.

The sequence of these two purifications can be reversed without endangering the yield of colchicine. It has also been found possible from time to time to eliminate either one of the two purifications, filtering or defatting. The only criterion as to how much purification of the

aqueous phase is necessary is the condition of the latter after initial purification. If the solution seems to clear and is of fairly light color, the colchicine can be extracted at once with chloroform.

The aqueous liquor, which by this time is about 300 gal, is placed in a clean mixing tank and 15 gal of chloroform or ethylene dichloride are added. This is mixed thoroughly for ½–¾ hr and is then allowed to settle for one hour or more, until a fairly sharp separation can be effected. The solvent phase is separated and set aside, and extraction of the aqueous phase is continued with 10 gal of solvent as described above. Emulsions can be separated by filtering them with filteraid, and washing the dried filtercake with solvent.

Extraction of the aqueous phase is repeated four to five times with 10 gal of solvent each time (calculated for about 300 gal of aqueous liquor representing 4000 lb of crude drug). The progress of the extraction cannot be judged by qualitative or quantitative tests on the aqueous phase, but only by the amount of residue in the solvent extraction. That is to say, that a sample of the chloroform or ethylene dichloride upon evaporation should leave behind only a negligible amount of residue. When that point is reached, the extraction can be considered finished and the aqueous liquor can be discarded. As a rule, and depending upon the efficiency of the mixing, 4–6 extractions will be found necessary.

The chloroform or ethylene dichloride extractions are combined and dried by mixing with anhydrous sodium sulfate at the rate of ½ lb per gal of solvent. Mix thoroughly, allow to stand for one hour, and filter through a clean stoneware filter. Collect the clear filtrates in a clean, stainless steel or glass-lined still. Wash the filtercake with fresh ethylene dichloride or chloroform until the washings become colorless, and add the washings to the main batch in the still.

Two-thirds of the solvent can now be evaporated under normal pressure; the rest should be removed under vacuo until most of the solvent has distilled off. The sticky and gummy residue will weigh about 30–35 lb, calculated for 4000 lb of average crude drug. Ordinarily, this material is now ready for the final crystallization.

If the original crude drug has been of especially poor quality, an additional step of purification should be included. The gum is dissolved in 15–20 times its weight of cold water, and the solution can be treated with a small amount of activated charcoal. It is then extracted again with chloroform or ethylene dichloride as described before. The solvent extractions are again combined, dried, filtered, and evaporated. The resulting resinous gum will now be considerably lighter in color.

The gummy residue of crude colchicine is dissolved in about 4–5

times its weight of cold water, that is, about 120 lb of water for the 30 lb of crude colchicine obtained from 4000 lb of average drug. The solution is heated to about 60°, and approximately one-third of the water is now taken off under vacuum. Care must be taken, and the still will need constant watching so that none of the content foams over. Distillation is continued under vacuum at 60–70° until all the chloroform has been removed.

Removing the last traces of chloroform is quite important, because *Pharmacopœia* specifications will not permit the presence of traces of chloroform in the finished product.

Another method of removing the chloroform consists in suspending the colchicine-chloroform gum in water and blowing steam through until all the chloroform has been removed. The disadvantage of this method lies in the fact that colchicine is less soluble in hot water than in cold.

The aqueous solution is now removed from the still and placed in a stainless steel or stoneware container in the icebox, where it is allowed to crystallize for several days. It is then filtered off, washed with a very small amount of water, and is dried under vacuum at 50°.

Filtrate and washings are combined, evaporated to one half their volume under vacuum at room temperature, and are then allowed to crystallize. Filtrate and washing from the second crop can be added to the aqueous phase of the next batch prior to extraction. Or, if all the colchicine has been processed, the aqueous liquors from the second crop can be extracted with chloroform or ethylene dichloride and the solvent can be worked up as described previously.

Another method of removing the chloroform consists in dissolving the colchicine-chloroform gum in two to three times its weight of ethyl acetate, and distilling that solvent until all the chloroform, whose boiling point is lower than that of ethyl acetate, has been removed. Upon cooling, the colchicine will crystallize from the ethyl acetate solution in anhydrous needles. This method yields the cleanest product, but the yield is somewhat reduced owing to the solubility of colchicine in the solvent. Further amounts of colchicine can be recovered from the motherliquors after concentration.

Colchicine salicylate is made by intimately mixing approximately molecular proportions of colchicine (74 parts) and salicylic acid (26 parts) in a ball mill.

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

The main use of colchicine lies in the treatment of gout, acute attacks of which can be shortened considerably and lessened in intensity by

the administration of colchicine until gastro-intestinal disturbances appear. Prophylactic administration of colchicine has also been tried with some success and without the appearance of toxic symptoms after two years.[15] Colchicine does not cure gout, but only relieves its symptoms, and it is of little or no value in other forms of arthritis.

When taken internally, colchicine is a local irritant. A strong solution, applied locally to subcutaneous or conjunctive tissue, produces irritation, hyperemia, and some sort of anesthesia. As mentioned before, the administration of colchicine must be discontinued upon the onset of diarrhea. Overdoses result in attacks of "strangling" nausea and burning of the mouth and throat, followed by vomiting, colic, and prostration, which is due mainly to its paralyzing influence upon the spinal motor and vasomotor nerves. Besides its effect on certain nerves, colchicine has an irritating and dilatory action on the capillaries, and its administration is often followed by leukopenia and leukocytosis. Colchicine is discharged into the gastro-intestinal tract, whose mucous membranes become quite inflamed. The secretion of urine can either be increased or diminished; the flow of bile is usually slightly increased.

Similar to tetanus, colchicine is less toxic to cold-blooded animals than to warm-blooded ones. It is quite stable against bacterial decomposition in putrefaction.

It was thought at one time that colchicine increases the rate of cell division in plants and animals. Actually this phenomenon is due to the power of colchicine to arrest cell division in the metaphase, as has been proved after hypodermic injection in mice.[16] In plants colchicine inhibits the formation of spindle fibers during cell division, resulting in a doubling of the number of chromosomes and a change in the genetic behavior.

The susceptibility of cancer cells to X-ray treatment has been shown to be increased, and in rats certain carcinomas have been healed or stopped and degenerated by the injection of distilled water, following a treatment with colchicine. However, this connection of cancer and colchicine is as inconclusive as the latter's use in the treatment of leukemia.

Colchiceine has been shown to have properties quite similar to those of colchicine, but its activity is only $\frac{1}{200}$ to $\frac{1}{40}$ of that of the latter.

ANALYTICAL AND TOXICOLOGICAL METHODS

Colchicine gives a yellow color with sulfuric acid which turns red on heating. It also gives a yellow color with Erdmann's reagent, with benzaldehyde-sulfuric acid and with selenic acid-sulfuric acid, the latter turning brown on heating. Froehde's reagent gives a blue color. Pre-

cipitates are obtained with tannic acid, phosphomolybdic acid, iodine-potassium iodide, and potassium-bismuth iodide, but Mayer's reagent and picric acid give a precipitate from a concentrated solution only.

Microchemical identification can be undertaken according to Klein and Pollauf.[17] The crude drug is extracted with chloroform and ammonia, and the resulting chloroform-colchicine compound is decomposed in boiling water to yield a pure, aqueous solution of colchicine. The reagent is platinum-rhodanide, made by mixing solutions of 5% platinum chloride and 5% potassium rhodanide, followed by filtration. In dilutions from 1:1000 to 1:250,000 small yellow crystals appear, formed by three lancets growing out of one central point. The difference between these crystals and those of the reagent can easily be ascertained.

For toxicological investigations, the stomach, intestines, and excrements are usually used. They are ground, extracted with alcohol, the alcohol is evaporated, and the residue is defatted with petroleum ether. Colchicine is then detected in the chloroform extractions of the aqueous phase.

Colchiceine, in hydrochloric acid solution, gives a green to black coloration with ferric chloride (aldehyde), which cannot be obtained with colchicine. Colchiceine is precipitated from an acid solution by bromine water, iodine-potassium iodide, mercuric chloride, and cadmium iodide. Lead acetate and copper acetate in aqueous solution give a white and green-yellow precipitate with colchiceine respectively.

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CHAPTER VII

THE CURARE ALKALOIDS

Although the sources of the "curare-alkaloids," the so-called pot, tube and calabash curares vary greatly with respect to both origin and content of active material, and despite our scant knowledge of the botanical origin, considerable inroads have been made into the chemistry and technology of these alkaloids.

The crude curares are thought to be prepared by the Indians of South America by extracting parts of one or more species of plants, often mixing different extracts and sometimes even adding other poisons, such as snake venom. Because of these uncertainties of sources and their inaccessibility, it is convenient to discuss the curare alkaloids in groups arranged according to their appearance in commerce in pots, tubes, or calabash. Definite relationship has been established, however, concerning the respective possible sources of tube and pot curare by isolation of identical or closely related active principles from both, thus also yielding a possible clue concerning their botanical origin. Included also in this chapter are, by necessity, the alkaloids contained in Pareira brava (*Chondrodendron platyphyllum*), one of which, bebeerine, has been stated to be identical with *l*-curine.

Wherever the Indians have been civilized, manufacture of curare has been discontinued, but through custom and necessity many wild tribes still prepare their extracts. In the regions of the upper Amazon the probable sources are the Strychnos species, such as *Strychnos castelnaea* or *Strychnos yapurensis*, while in the regions of the Peruvian Amazon *Chondrodendron* species might be the main source, with or without the admixture of some Strychnos species. The Indians of the upper Orinoco, of Venezuela (Esmeralda), of the upper French Guiana and those of British Guiana are all reported to use mainly a different species of Strychnos. Much has been written by travelers about the preparation of the curares,[1] which, according to Vellard,[2] takes place in three steps: The extraction of the root or bark with water, a short period of boiling to precipitate impurities which can then be strained off or the clear liquid decanted, and a final slow evaporation under mild heat. The final extract is a thick paste, brownish to black, more or less solidified according to its age, shiny and resinous, with a more or less agreeable

aroma and an intensely bitter taste. According to Dutcher,[3] the material is best prepared for storage by drying and milling. Curare is soluble in cold water and dilute alcohol. According to Gold,[4] curare can be purified by dissolving it in alcohol, filtration, removing of the alcohol under vacuum, dissolving the residue in water, and adding acetone until precipitation starts. The precipitate is inert material.

Tube Curare

This material is also known as para or bamboo curare because it is stored in hollow bamboo canes. From this material Boehm [5] first isolated crystalline curine and amorphous tubo-(or para-)-curarine, the latter having since been obtained in crystalline form by a number of investigators. *d*-chondocurine and *d*-isochondrodendrine have also been obtained recently.[5,8]

Relationships have been established between curine, tubocurarine, and bebeerine, as will be shown later, and because of these correlations, the botanical origin of tube curare has been established as coming at least partially from the family of Menispermaceae and probably from one or more species of *Chondrodendron*, called *Chondodendron* by Ruiz and Pavon. King [7] has undertaken extensive experiments to determine the origin of bebeerine, the laevorotatory form of which was obtained from *Chondrodendron platyphyllum*, while the dextrorotatory isomer was found in *Chondodendron microphyllum*. A species examined by King [8] was found to contain *l*-curine and *l*-tubocurarine chloride, but Dutcher,[3] working with apparently the same botanical species, obtained *l*-curine and *d*-tubocurarine chloride. There is, therefore, the possibility of the existence of a not yet defined species of *Chondrodendron*, but the family of the drug has been definitely established as such.

l-Curine, $C_{36}H_{38}O_3N$, can be crystallized from benzol with one mole of that solvent. The melting point of the solvated material is 161°; the melting point of the anhydrous form is 222°. The anhydrons form can be obtained by crystallization from methanol. Its rotation in pyridine is α_D −328°. Both the hydrochloride and dihydrochloride are crystalline, whereas the yellow platinichloride is amorphous.

Boehm, in his initial investigations,[5] stipulated the formula $C_{18}H_{19}O_3N$, but this mistake was soon rectified by Spaeth, Leithe, and Ladeck [9] and by Spaeth and Kuffner.[10] Boehm also noted the presence of a methoxyl and of a phenolic hydroxyl group. Fusion with potassium hydroxide yielded protocatechuic acid. Dry distillation as well as distillation with zinc dust or with soda lime gave trimethylamine and a substance thought to be *p*-hydroxy-quinoline-methyl ether.

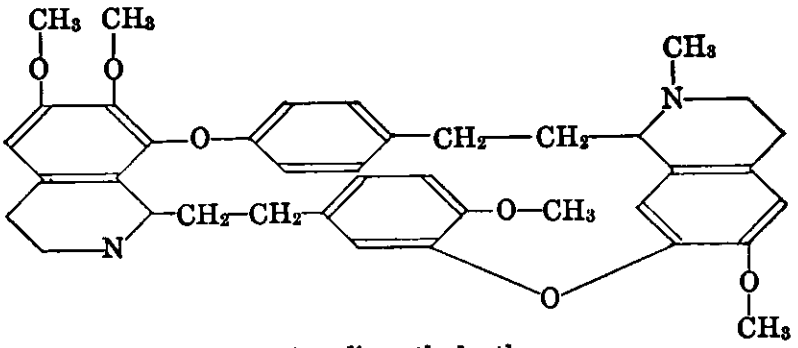
Spaeth, Leithe, and Ladeck not only established the identity of *l*-curine

with that of *d*-bebeerine,[9] but they also suggested a working formula for that compound.

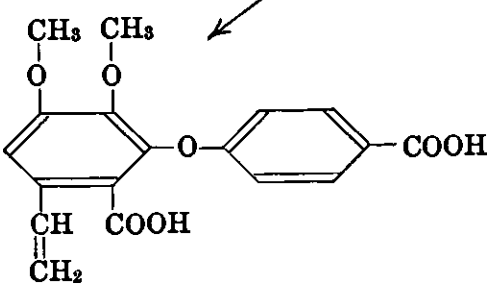
l-Bebeerine was first prepared by Scholtz [11] from commercial bebeerine (*Nectandra rodioei* Hook; *Chondrodendron platyphyllum* St. Hil. Miers.), and the same investigator also prepared the dextro and racemic forms of that alkaloid. The melting points of the racemic forms of bebeerine and of a mixture of equal amounts of *d*- and *l*-curine were found to be identical.[12] Scholtz also assumed the simple formula for bebeerine ($C_{18}H_{19}O_3N$), and on that basis he found one methoxyl, one hydroxyl and methylimino group present. From the general reactions, the presence of a methyltetrahydroisoquinoline nucleus was assumed. King [13] and Spaeth and Kuffner [14] stipulated the presence of the bis-benzylisoquinoline nucleus, now generally accepted. Spaeth and Kuffner, on partial methylation of curine, obtained a mixture of a dimethyl and two monomethyl ethers, one of which was obtained in a crystalline form ($C_{37}H_{40}O_6N_2$, m.p.: 208°). Complete methylation furnishes O-methylbebeerine metho-salts,[15] which can be degraded according to Hofmann to a mixture of three methine-bases (O-methylbebeerinemethine methiodides, melting points: 190°, 230°, and 234°). Further degradation under reducing conditions gave trimethylamine and a substance (O-methylbebeerilene, $C_{36}H_{32}O_6$, m.p.: 199°) (or $C_{36}H_{40}O_6$, m.p.: 187°),[16] which can be oxidized to form 2,3-dimethoxy-5,6,4′-tricarboxydiphenyl ether.[14] This substance had not only been obtained by Faltis and Neumann from isochondrodendrine (see later),[17] but it had also been synthesized by them and its structure was thus confirmed.

The formula suggested by Spaeth and Kuffner [14] has been confirmed indirectly by King in his investigation of the relationship of bebeerine methochloride to tubocurarine chloride. Faltis, Kadiera, and Doblhammer [18] ozonized dimethylbebeerine-methine and, on hydrogenation of the product, obtained two acids: 2,3-dimethoxy-6,4′-dicarboxy-5-vinyldiphenyl ether (which had also been obtained from isochondrodendrine), and 2,6′-dimethoxy-5,3′-dicarboxy-4′-vinyldiphenyl ether. The latter on decarboxylation and oxidation gave 2,6′-dimethoxy-4-carboxydiphenyl ether, whose constitution was confirmed by synthesis.

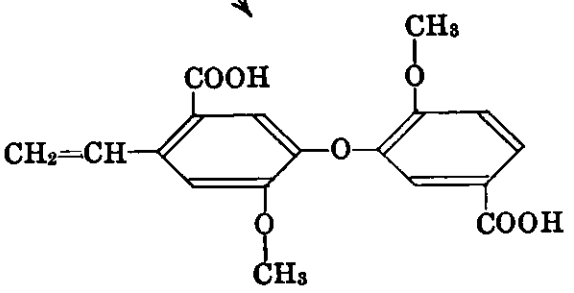
The structure of bebeerine and curine was thus established with exception of the relative position of the methoxyl and hydroxyl groups, and that problem was solved by King,[19] who ethylated, and thus marked, the hydroxyl groups. Degradation gave O-ethylbebeeriline, which corresponds to the O-methylbebeeriline mentioned previously, and which could be oxidized with potassium permanganate, first in acetone and then in water, to yield two isomeric acids, $C_{18}H_{16}O_9$, the constitution of which was confirmed by synthesis.



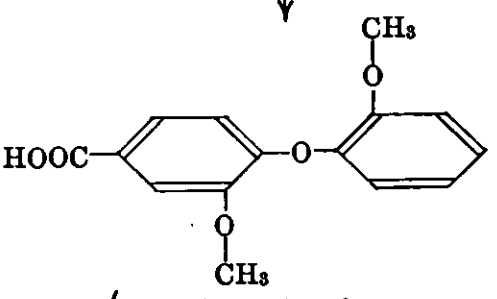
Curine-di-methyl ether



2,3-Dimethoxy-6,4′-di-carboxy-5-vinyl-diphenyl ether



2,6′-Dimethoxy-5,3-di-carboxy-4′-vinyl-diphenyl ether



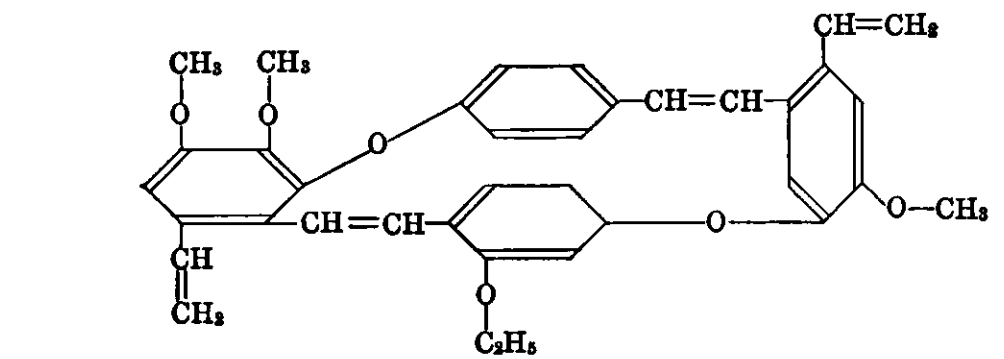
2,6′-Dimethoxy-4-carboxy-diphenyl ether

Tubocurarine chloride was first obtained by Boehm [5] in an amorphous state, but in 1935 King [15] succeeded in crystallizing the quaternary salt. Since then, the laevorotatory form has been isolated by King [8] from *Chondrodendron tomentosum* Ruiz and Pavon, but Wintersteiner and Dutcher [6] and Dutcher [3] have obtained the dextrorotatory form from the same species. The latter form can be recrystallized from water, but the crystallizates obtained contain various amounts of water of crystallization which is hard to remove upon prolonged drying in vacuo at

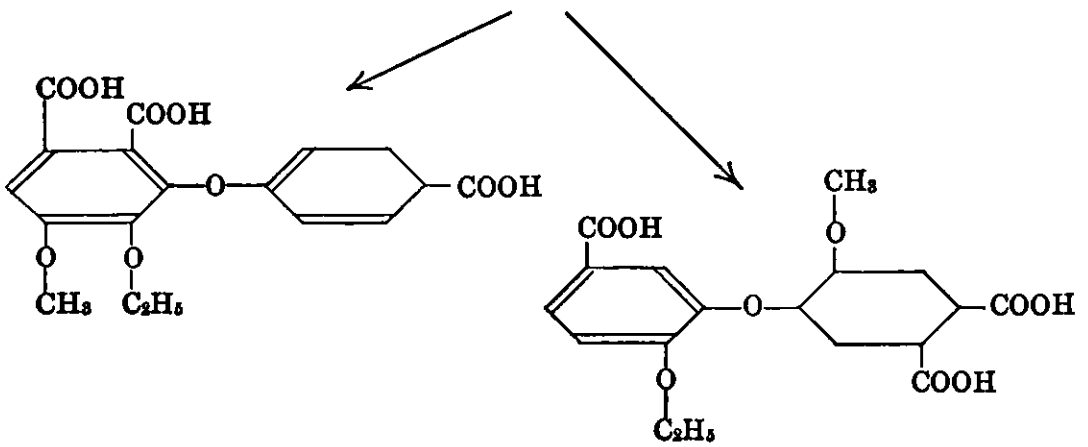
elevated temperatures. The pentahydrate shows m.p.: 268–269° with previous softening; the anhydrous material has m.p.: 274–275°.[3] Values for the optical rotation have been recorded by Dutcher (*loc. cit.*) and by King.[20] The material is somewhat more soluble in ethanol and methanol and insoluble in pyridine, chloroform, benzene, and acetone. No other salts were obtained in crystalline form by Dutcher (*loc. cit.*).

The relationship of *d*-tubocurarine chloride to *l*-curine methochloride was established by King,[15] but the relative positions of the methoxyl and hydroxyl groups is not yet definitely known. They are probably diastereoisomers, and, although their optical rotation is in opposite directions, they are not enantiomorphs. *d*-Bebeerine methochloride is enantiomorphic with *l*-curine methochloride, but it differs from *d*-tubocurarine chloride by having two dextrorotatory centers of asymmetry, while the latter contains one dextro- and one laevorotatory center of asymmetry.

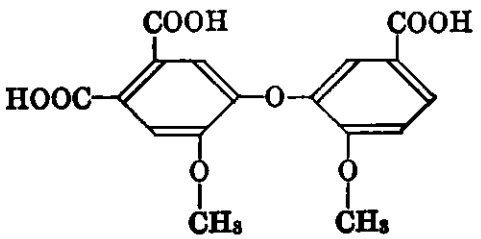
Methylation of tubocurarine chloride, followed by degradation, gave four methine bases, isolated as their respective methiodides. Three of these were identical with the three O-methylbebeerinemethine methiodides described previously. Further degradation likewise gave trimethylamine and O-methylbebeerilene, $C_{36}H_{32}O_6$.



O-Ethylbebeeriline



By oxidation of O-methylbebeerilene, King [21] obtained three acids, two isomeric ones ($C_{17}H_{14}O_9 \cdot 2H_2O$), one of which was 5,6,4′-tricarboxy-2,3-dimethoxydiphenyl ether, already obtained by Spaeth and Kuffner [10] and by Faltis and Neumann [22] and identified by Faltis *et al.*[23] The second of the isomeric acids was given the following constitution,



but two possible formulae were suggested for the third acid, $C_{34}H_{30}O_{16}$.

$(MeO)_2{=}C_6H(COOH)_2{-}O{-}C_6H_4{-}CH(OH){-}CH(OH){-}C_6H_2(OMe)(COOH){-}O{-}C_6H_3(OMe)(COOH)$

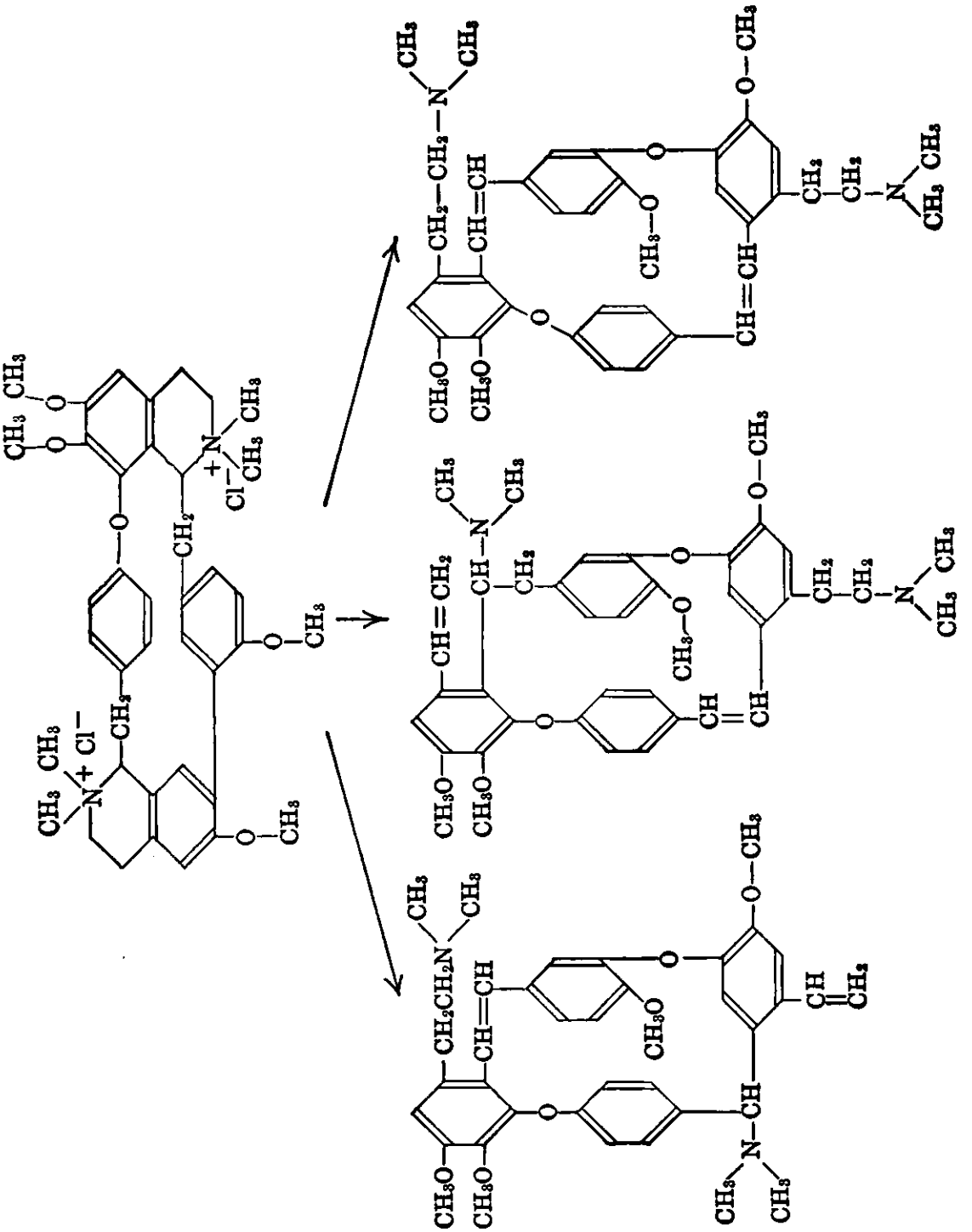
$(MeO)_2{=}C_6H(COOH)(OC_6H_4COOH){-}CH(OH){-}CH(OH){-}C_6H_3(OMe){-}O{-}C_6H_2(OMe)(COOH)_2$

The formula for O-methyltubocurarine chloride, identical with O-methylcurine methochloride, can give four possible optically active forms, two laevo and two dextro, because of the presence of two asymmetric centers adjacent to the two nitrogen atoms. Each one of these four optically active compounds can give rise to four methines, depending upon the position of fission in relation to the nitrogen atoms. The possible forms of these methines are shown in the following diagram. Because a limited number of methine bases was obtained, the above-mentioned laevo-laevo and laevo-dextro configuration was stipulated by King.[24]

From his specimen of *Chondrodendron tomentosum* Ruiz and Pavon, Wintersteiner and Dutcher,[6] and Dutcher [3] obtained two more tertiary bases, *d*-chondocurine and *d*-isochondrodendrine.

d-Chondocurine was found to be a new member of this group of alkaloids and to have the formula $C_{36}H_{38}O_6N_2$. The base was obtained from an ether extract and was recrystallized from methanol, m.p.: 232–234°; α_D +200° (HCl), hydrochloride m.p.: 280–282°. The sulfate was also crystallized from water, m.p.: 263–265°, α_D +193° (H_2O). A methanolic solution of the free base gave a faint pink color with ferric chloride; an aqueous solution of the hydrochloride gave a positive Millon's reaction.

d-Isochondrodendrine, $C_{36}H_{38}O_6N_2$, has been prepared previously by Scholtz [11] and by Faltis [18] from the root of *Chondrodendron platyphyllum*. The base was crystallized by Dutcher from chloroform and methanol, m.p.: 305° (decomp.), α_D +120° (HCl); the sulfate, previ-

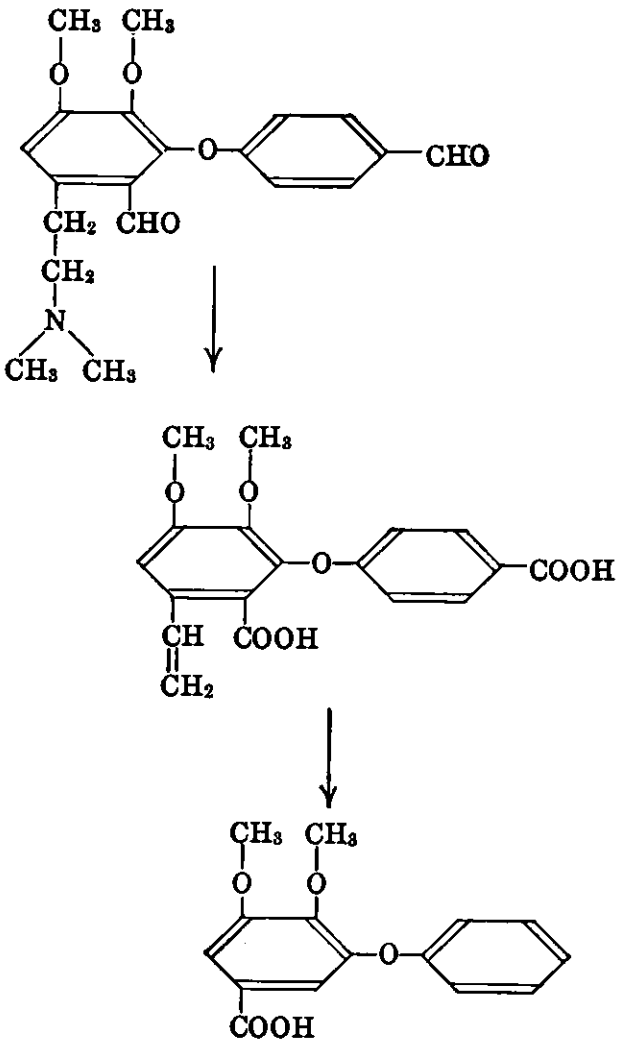


ously prepared by King [25] crystallized as the heptahydrate, m.p.: 280° (anhydr.), hydrochloride m.p.: 282–284°.

The formula of this alkaloid was determined in 1932 by Faltis, Wrann, and Kuehas.[26] The phenolic base, obtained by demethylation with hydrochloric acid, was found to give a positive catechol reaction.[27] Exhaustive methylation and degradation gave trimethylamine and a

substance, $C_{36}H_{32}O_6$,[28] which on oxidation with potassium permanganate furnished an acid, m.p.: 178°, which was synthesized by Faltis and Frauendorfer[29] and was identified as 2,3-dimethoxy-5,6,4′-tricarboxy-diphenyl ether.

Isochondrodendrine yields two methine bases in the course of the Hofmann degradation,[30] one of which, on ozonization followed by hydrogenation yields 2,3-dimethoxy-6,4′-dialdehyde-5-dimethylaminoethyl-diphenyl ether. Oxidation of the methochloride of that compound gave a good yield of 2,3-dimethoxy-6,4′-dicarboxy-5-vinyl-diphenyl ether,[31] indicating the presence in the molecule of two identical halves. Decarboxylation, followed by oxidation, gave an acid, 2,3-dimethoxy-5-carboxydiphenyl ether, which was also synthesized.[31]



Pot Curare

This form is said to be no longer available in commerce, but some specimens have been obtained from time to time. The material is a

dark brown, almost dry extract, packed in brown or grayish-brown, unglazed, earthenware pots. Substitutes and adulterants are often found in small, glazed pots.

King,[32] who examined a specimen of pot curare, discussed its possible botanical derivation at great length, and he came to the conclusion that his particular sample probably originated from a Menispermaceae species or from a mixture of Menispermaceae and Strychnos. *Strychnos castelnaea* Wedd. was ruled out by him, however. On the other hand, one of the active principles of pot curare, neoprotocuridine, can be thought of as an internally compensated centrosymmetrical form of isochondrodendrine, thus indicating a close relationship of tube and pot curare. Furthermore, both protocuridine and curine can be thought of as condensation products of coclaurine, an alkaloid isolated from *Coccolus laurifolius* D.C., natural order of Menispermaceae, the former by symmetrical and the latter by asymmetrical condensation.[33] Although this argument in itself does not constitute any proof, it does tend to confirm King's (*loc. cit.*) findings.

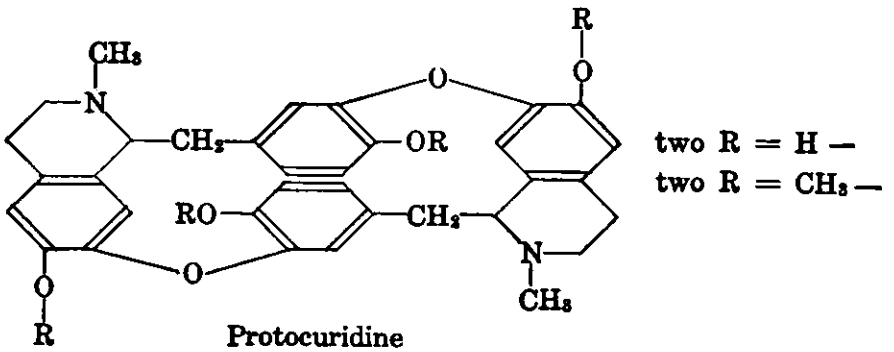
This form of curare was also examined by Boehm,[34] who obtained from it three alkaloids, protocurine, $C_{20}H_{23}O_3N$, m.p.: 306° (dec.), protocuridine, $C_{36}H_{38}O_6N_2$, m.p.: 275°, and protocurarine, $C_{19}H_{23}O_2N$, which is its most toxic constituent. Protocuridine was found to be only slightly soluble in most solvents, but the amorphous protocurarine is easily soluble in water and gives a color reaction which is characteristic for the alkaloids of Strychnos species.

King [32] separated the alkaloids into a quaternary and a nonquaternary fraction. Precipitation with sodium bicarbonate and extraction with ether gave the tertiary alkaloids, protocuridine and neoprotocuridine, neither of which is pharmacologically very active. The quaternary bases were concentrated and purified by fractional precipitation with mercury bichloride.

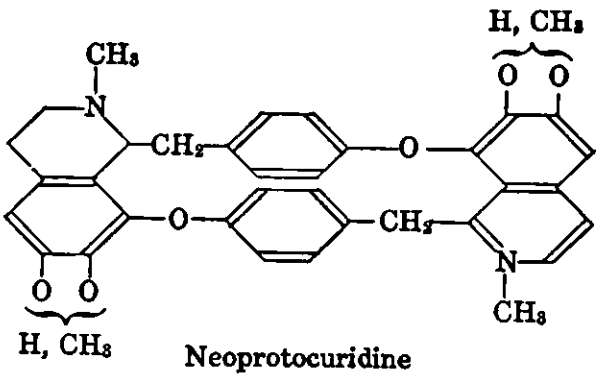
One of the tertiary alkaloids isolated by King is protocuridine, the hydrochloride of which was recrystallized from water (m.p.: 295°). The base was obtained in crystalline form by precipitation with sodium bicarbonate, and it was then recrystallized from pyridine and water (m.p.: 295°). It was found to contain two hydroxyl and two methoxyl groups, and to resemble the tube curare alkaloids in giving a positive Millon reaction. On methylation the base yields O-methylprotocuridine methiodide, $C_{40}H_{48}O_6N_2I$ (m.p.: 318°).

Neoprotocuridine, an isomer of protocuridine, was separated from the latter by means of its sparingly soluble hydrochloride (m.p.: above 310°). The base was obtained in crystalline form by precipitation with sodium bicarbonate (m.p.: 232°). Methylation gave O-methylneoproto-

curidine methochloride, which by the Hofmann degradation gave a methine, which was methylated to methylneoprotocuridine-methine-methiodide. This substance was identical with one obtained in the same manner from isochondrodendrine. Neoprotocuridine is, therefore, thought to be an internally compensated form of isochondrodendrine, but the relative positions of the hydroxyl and methoxyl groups are not known. The following formulae have been suggested for protocuridine and neoprotocuridine:



Protocuridine



Neoprotocuridine

Little is known of the alkaloids of the quaternary fraction, from which a small amount of a very toxic phenolic iodide could be isolated. The presence of other bases was demonstrated by King (*loc. cit.*) by the preparation of three fully methylated methiodides, melting at 260, 295, and 318°.

Calabash Curare

This material is exported in small gourds and is often called gourd curare for that reason. This drug is often collected in Venezuela or the upper Orinoco, and it is quite certain that it is made almost exclusively from different Strychnos species, one of which has been definitely identified as *Strychnos toxifera* Schomb. King [35] was able to isolate from an authenticated sample of that drug a base which was found to be identical with curarine, also known as toxiferine. He examined other Strychnos species collected in the same territory, and, although they contained

alkaloids, these were not of the curarine type. Strychnolethaline and curalethaline have been isolated from *Strychnos lethalis*,[86] but most of the curarine-like bases have been isolated from *Strychnos toxifera*. Karrer and Schmid [87] have been able to correlate alkaloid content of calabash curares with their geographic and botanical origins, as shown in Table 7.1:

TABLE 7.1

Origin	Formula	Name
Strychnos toxifera	$C_{20}H_{23}N_2^+X^-$ $C_{20}H_{21}N_2^+X^-$	Toxiferine—II Toxiferine—I
Colombia and Venezuela	$C_{20}H_{23}N_2^+X^-$ $C_{20}H_{23}N_2^+X^- \cdot H_2O$ $C_{20}H_{23}N_2^+X^-$ $C_{20}H_{21}N_2^+X^-$	Toxiferine—II C-Toxiferine—II C-Dihydrotoxiferine—I C-Curarine—I
Central Orinoco Venezuela No. Urbana	$C_{20}H_{21}N_2^+X^-$ $C_{20}H_{23}N_2^+X^-$ $C_{20}H_{21}N_2^+X^-$	C-Curarine—I C-Curarine—II C-Curarine—III

The quaternary alkaloids of calabash curare are best separated and purified by chromatographic adsorption. Many of the bases easily lose one mole of water, forming a tertiary base. Most of their salts are crystalline. Some of the alkaloids, such as C-(for calabash) curarine—I, easily dehydrate, forming an ether base. Wieland and his collaborators,

TABLE 7.2

Compound	$\alpha_D^{20°}$	M.p. Picrate	Potency (γ)
Toxiferine—I	−610	270	0.3
Toxiferine—II		216	5.0 as picrate
C-Dihydrotoxiferine—I	−605	185	1.5
C-Isodihydrotoxiferine—I	−550	242	3–4
Toxiferine—IIa	+66	210	20–30 as hydrate
Toxiferine—IIb	+78	216	100–150 as hydrate
C-Toxiferine—II	+72	215	10 as hydrate
C-Curarine—I	+73	above 300	3–4
C-Curarine—II	+74	204	50–100
C-Curarine—III	−930	189	.....

in a series of papers,[38] have described the isolation and the physical constants of a considerable number of bases, as described in Table 7.2.

MANUFACTURING METHODS

Although a few patents have been issued,[39] protecting manufacturing methods of curare alkaloids, relatively little is known concerning their technology.

Concerning the manufacture of *d*-tubocurarine chloride, the method described by Dutcher [3] may serve as an illustrative example:

350 grams of powdered "Serpa" curare was mixed intimately with 2 liters of 1% tartaric acid solution, and the mixture was shaken repeatedly. The insoluble material was filtered off and treated successively with one liter and then with 500 cc of 1% tartaric acid solution. The insoluble material was discarded. The combined aqueous acid solutions were then precipitated with lead subacetate, until a clear filtrate showed no further precipitation with lead subacetate. The material was filtered, and the filtercake was washed with acidulated water. The clear filtrates and washings were combined and freed from excess lead by treatment with hydrogen sulfide. After filtration, the pale yellow solution was concentrated under vacuum at a temperature not exceeding 40°. When the volume was reduced to about 3 liters, sodium bicarbonate was added, and the solution was thoroughly extracted, first with ether and then with chloroform. The solvents were dried and evaporated separately.

The aqueous alkaline phase was acidified immediately after the chloroform extractions were finished, and a solution of free reinecke acid was added until precipitation was complete. The pink precipitate was filtered and washed thoroughly with water. The dried reineckate was dissolved in dry acetone, and the solution was filtered. To the clear filtrate was added a solution of silver sulfate in order to precipitate all the reinecke acid as silver reineckate, and the sulfates of the quaternary bases so produced were converted into the corresponding hydrochlorides by adding sufficient barium chloride solution to convert all the sulfates into chlorides and also to remove any excess of silver sulfate. The total precipitate consisting of silver reineckate, silver chloride, and barium sulfate was filtered off and washed with a small amount of dilute acetone. The nearly colorless combined filtrates and washings were evaporated to dryness under vacuum, at a temperature below 40°. The residue was crystallized by adding a small amount of cold, distilled water. Approximately 3 grams of crystalline *d*-tubocurarine chloride were obtained. The residue from the motherliquor from this crystallization consisted

of practically pure *d*-tubocurarine chloride, as was shown by a bio-assay, but it could not be crystallized. By using a slightly different technique, picric acid can be used instead of the reinecke acid mentioned previously.

From the ether extraction of the aforementioned tertiary bases, *d*-chondocurine could be crystallized upon the addition of methanol to the residue. The yield of this base was approximately 2 grams.

The residue from the chloroform extractions was digested with hot methanol, and the mixture was allowed to crystallize. After filtration and recrystallization from chloroform and methanol, 6.5 grams of *d*-isochondrodendrine was obtained.

The foregoing method is also the subject of a patent.[40] It is similar to one published by King.[41] The preparation of a sterile solution of curare alkaloids has also been protected by patent.[42]

The alkaloids of calabash curare are usually purified by selective adsorption and elution of their reineckates from a column of aluminum oxide.[43]

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

Curare, when injected hypodermically, paralyzes the motor nerve endings of the voluntary muscles. In lethal doses death occurs because of respiratory failure. It is said to be considerably less toxic when taken orally. The lethal dose has been found to vary greatly,[44] probably owing to a varying content of alkaloids.

Most important is the lissive action of certain curares, which is probably due to *d*-tubocurarine, although other quaternary ammonium bases are known to exert similar actions.[45] Lissive action is defined as "the selective removal of pathological rigidities without apparent diminution of voluntary power." It is thus possible to control tetany in parathyroidectomized dogs,[46] but tubocurarine chloride is supposedly without action in that respect.[47]

Curine is said to be of a somewhat lower toxicity than curare, and is without the latter's peripheral action. It is primarily a central depressant, and in toxic doses death is due to heart failure.[48]

ANALYTICAL AND TOXICOLOGICAL METHODS

Methods of biological assay have been described by King [49] and by Dutcher.[50]

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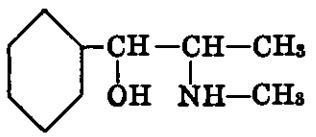
CHAPTER VIII

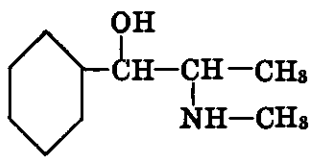
ALKALOIDS OF EPHEDRA

CHEMISTRY

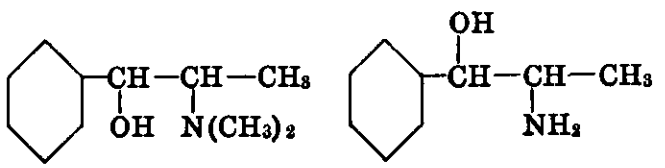
Ephedrine is the main alkaloid in a large number of plants of the species Ephedra, the best known of which are known collectively as Ma-huang.



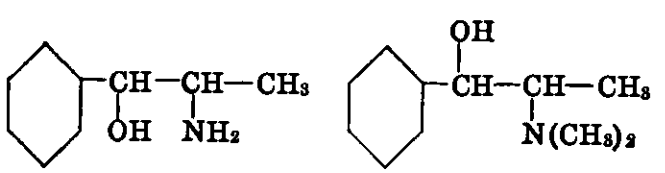
Secondary in most plants, but occurring as the main alkaloid in some species, is pseudoephedrine, an isomer of ephedrine.



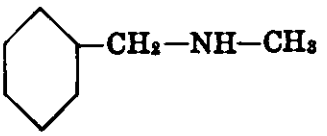
Two more bases were first prepared by Smith,[1] *l*-N-methylephedrine, and nor-*d*-pseudoephedrine.



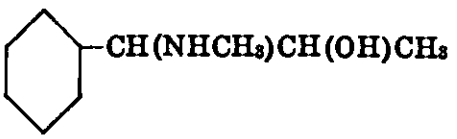
Nagai and Kanao [2] described two additional alkaloids, namely *d*-N-methyl-pseudoephedrine, and *l*-nor-ephedrine. Both alkaloids are found in certain European species.[3]



Other amines isolated from ephedra are benzylmethylamine, found by Chen *et al.*,[4] and ephedine, found by Chou and Mei.[5]



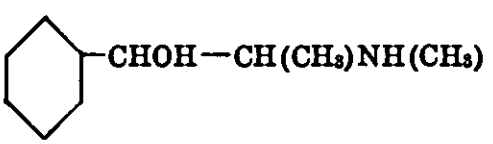
Isoephedrine, which has been mentioned in the literature several times, does not seem to occur in nature, and its constitution is still unknown. The formula below has been suggested by Fourneau and Benoit.[6]



Ephedrine was first isolated by Nagai in 1887, but its pharmacological properties and uses have been known to the Chinese for about 5000 years. Interest in the alkaloid and in the drug has mounted steadily since 1924, when Chen and Schmidt [7] discussed the therapeutic possibilities of the amine. A large amount of literature has appeared since then.

The structure of ephedrine and pseudoephedrine has been known for some time, but the complete stereochemical structure and relationships have only been established relatively recently.

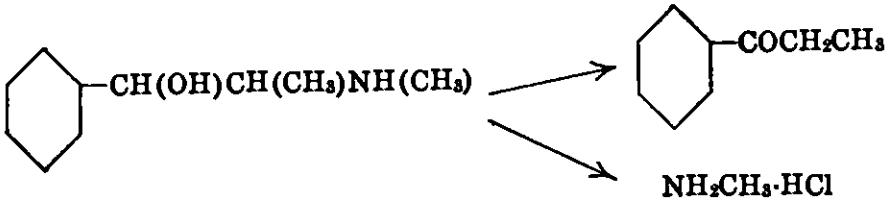
Ladenburg and Oelschlaegel [8] demonstrated the formation of a nitroso-derivative (m.p. 82°), confirming the presence of a hydroxyl group, and they also prepared a dibenzoyl-derivative (m.p. 120°), both from pseudoephedrine. The presence of an N-methyl group was proved by the formation of methylamine by degradation of the base with strong hydrochloric acid, and the simultaneous formation of homologues of methylamine indicated that the N-methyl group was not a terminal one situated at the end of a chain. Oxidation under suitable conditions gave either benzaldehyde or benzoic acid, proving the proximity of the hydroxyl carrying carbon to the benzene ring. On the basis of the information available, the following formula for ephedrine was suggested:



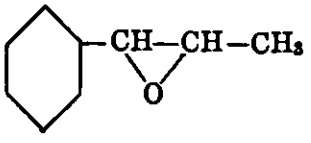
The close relationship of ephedrine and pseudoephedrine was established when it was found that the former could be isomerized to the

latter either by acylation, followed by hydrolysis,[9] or by boiling with hydrochloric acid.[10] The latter method was shown to be reversible and to give an equilibrium mixture.

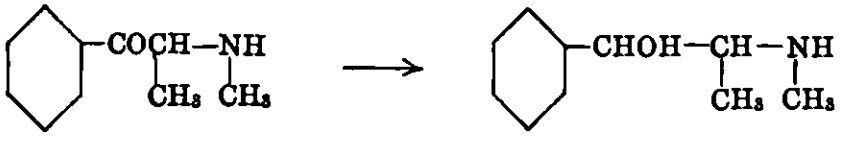
Support for the Ladenburg formula was obtained by Schmidt and Buemming, who subjected the hydrochloride of both ephedrine and pseudoephedrine to destructive distillation under carbon dioxide. They obtained, in both cases, propiophenone and methylamine hydrochloride.[11]



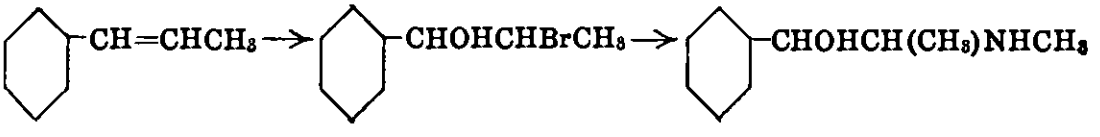
Miller [12] prepared a quaternary ammonium base by exhaustive methylation of ephedrine, which, on boiling in aqueous solution, gave an oil and trimethylamine. This oil, $C_9H_{10}O$ (b.p.: 205°, α_D +65.8°), was further examined by Rabe,[13] who identified it as being alpha-phenyl-propylene-alpha, beta-oxide.



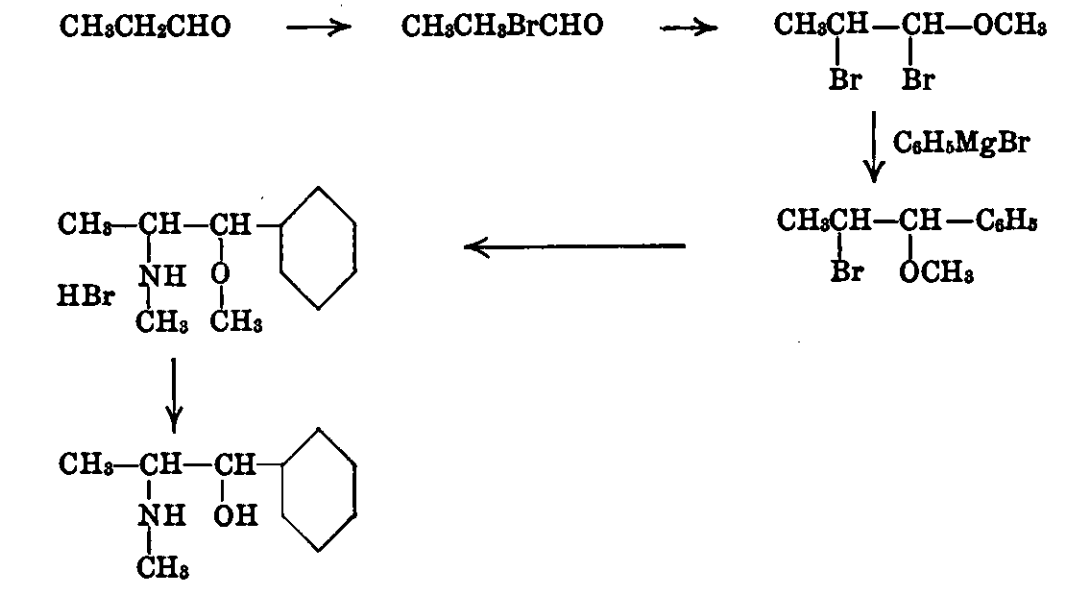
The structure of ephedrine and pseudoephedrine has been confirmed beyond any doubt by repeated syntheses, the first of which was achieved by Eberhard [14] by the reduction of alpha-methylamino-propiophenone.



The racemic forms of ephedrine and pseudoephedrine were thus obtained. Another synthesis was performed by Fourneau and Puyal [15] from phenylethylcarbinol through propenylbenzene and the bromohydrine.



A total synthesis and resolution into the optically active bases was first achieved by Spaeth and Goehring [16] through the following steps:



The racemic pseudoephedrine, which was initially obtained, was resolved through repeated crystallization of the tartrates into *d*- and *l*-pseudoephedrine, the former being identical with the naturally occurring one.

These optically active, *d*- and *l*-pseudoephedrines were finally isomerized into *l*- and *d*-ephedrine respectively, whereby the former proved to be identical with the naturally occurring compound.

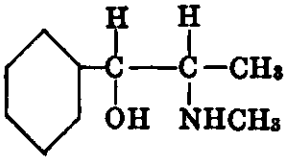
Syntheses which gave racemic ephedrine directly have been reported by Manske and Johnson [17] and by Skita and Keil,[18] who hydrogenated alpha-phenylpropane-alpha, beta-dione in alcohol in the presence of methylamine.

The racemic ephedrine thus formed (a little racemic pseudoephedrine was also obtained) was resolved into its antipodes by crystallization of the *d*- and *l*-mandelic acid salts.

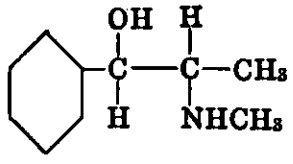
A pseudosynthesis, which yields *l*-ephedrine directly, is now being used on a commercial scale. Fermenting sucrose, together with benzaldehyde, yields the laevorotatory *l*-phenylpropanol-1-one-2, which, upon reduction in the presence of methylamine, yields *l*-ephedrine. Details of the process will be described in another section of this chapter.

Inasmuch as ephedrine has two asymmetric carbon atoms, four stereoisomeric forms are theoretically possible, and, as indicated before,

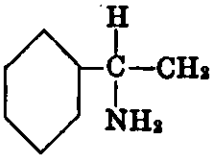
all four forms are known. The orientation of the different substituents has been definitely established by Emde,[19] by Freudenberg *et al.*,[20] and by Leithe,[21] who likened the configuration of the carbon carrying the hydroxyl group to the configuration of dextro- and laevo-mandelic acid. The contribution of this carbon to the total optical rotation is negative in *l*-ephedrine and positive in *d*-pseudoephedrine, and can be correlated to laevo-mandelic acid in the former and to dextro-mandelic acid in the latter. Both compounds can be reduced to the same desoxy-ephedrine, and it was shown that the carbon carrying the methylamino group contributes positively to the total optical rotation. The configuration about this second center of assymetry has been investigated by Leithe (*loc. cit.*) and by Freudenberg and Nikolai,[22] the former correlating it to *l*-(−)-alphaphenylethylamine and the latter drawing the analogy to the naturally occurring *l*-(+)-alanine.



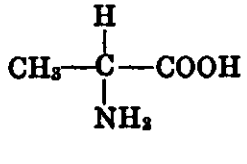
l-Ephedrine



d-Pseudoephedrine



l-(−)-α-Phenetamine



l-(+)-Alanine

The isomerization of *l*-ephedrine to *d*-pseudoephedrine by boiling with hydrochloric acid, a process which is reversible and which slightly favors the direction indicated here, is thought to take place through an intermediate chlorcompound, formed by the substitution of the hydroxyl group by chlorine. This chlorocompound is thought to rearrange upon hydrolysis.[23]

Another possible way of isomerization is by acetylation, followed by hydrolysis. A recent investigation by Welsh [24] has greatly clarified this reaction. He reported that both acetyl-ephedrine and acetyl-pseudoephedrine have an N-acetyl or amide structure and not, as previously reported, an O-acetyl or ester structure. Both compounds, under suitable conditions, form stable HCl-addition products, which rearrange to the O-acetyl hydrochlorides on heating. During this rearrangement, no appreciable inversion occurs on the carbon alpha to the phenyl group

in the case of pseudoephedrine, but the ephedrine derivative under the same condition yields a mixture of the diastereoisomers with pseudoephedrine predominating in the ratio 38:62. It was found in the course of the investigation that the inversion occurs during the shift of the acetyl group from the nitrogen to the oxygen, and that alkaline hydrolysis of the acetyl derivatives produces no inversion. As a matter of fact, in the presence of alkali the O-acetyl-hydrochlorides rearrange quantitatively into the N-acetyl derivatives without a change in configuration. Welsh (*loc. cit.*) determined that the speed of isomerization is relative to the hydrogen ion concentration and is almost instantaneous at a high pH.

l-Ephedrine is found in commerce, as a rule, as the hemi-hydrate (m.p.: 40°); occasionally the anhydrous form is obtained (m.p.: 38.1°). The base is colorless, and boils at 225° at ordinary pressure or at 152–153° at 25 mm Hg. Its rotation is −6.3 in alcohol or +11.2° in water. The base is readily soluble in water as well as in most organic solvents, including mineral oil. The salts with mineral or organic acids are colorless crystalline solids, usually soluble in water and almost insoluble in alcohol. Ephedrine base, when kept in solution in chloroform for some time, is gradually turned into the hydrochloride, which crystallizes out.

d-Pseudoephedrine can be crystallized from ether in rhomboids (m.p.: 118–119°), with an optical rotation of +51.2° in alcohol. The base is readily soluble in alcohol ether and other organic solvents such as benzol, but it is only slightly soluble in water. The salts are crystalline and are soluble in water and somewhat more in alcohol than the salts of *l*-ephedrine. The hydrochloride is soluble in acetone and chloroform, thus making possible the separation from the hydrochloride of laevo-ephedrine.

l-nor-Ephedrine can be crystallized from a mixture of ether and pentane, but it is often obtained as a more or less semicrystalline mass. Most of its salts are crystalline.

nor-*d*-Pseudoephedrine has also been isolated from *Catha edulis*.[25] It is somewhat soluble in water, but can be extracted with chloroform and recrystallized from benzol (m.p.: 77–78°). Its constitution has been confirmed by synthesis. Its salts are crystalline, and salts and the base are dextrorotatory.

N-methyl-ephedrine and the corresponding *d*-N-methyl-pseudoephedrine can be found in the motherliquors from the preparation of *l*-ephedrine. Both bases can be crystallized from methanol, the former m.p.: 88° and the latter m.p.: 30°. The salts of both alkaloids are crystalline and soluble in water.

TABLE 8.1

	l-Ephedrine	*d*-Pseudo-ephed-rine	*l*-nor-Ephedrine	nor-*d*-Pseudo-ephed-rine	N-methyl-ephedrine	d-N-methyl-Pseudo-ephedrine	Ephe-drine
Base m.p.....	38.1(40)	119	51	78	88	30	87
Base b.p.....	225		168/22 mm		139/14 mm	145/24 mm	
Base α_D^{20}....	−6.3(alc.) +11.2(H_2O)	+51.2		+38°	−29.2	+48.1	
HCl.....	221	182	173	179	190		90
HCl α_D^{20}.....	−34	+62	−33.1	+42 +53	−29.8	+58.1	
H_2SO_4.....	243		286	295			
Oxalate.....	250	218	245	235	187		
Aurichloride...	131	128	188		129	127	
Platinichloride	186		221				
Acid tartrate..			160	151		152	
Picrate.....					144	153	190

NATURAL SOURCES

Natural ephedrine is almost entirely produced from one or more of the Chinese species of ephedra known under the collective name of Ma-huang. The genus ephedrine belongs to the family of Gnetaceae, the members of which are found all over the world wherever there is a warm and dry climate, especially in certain sections of Asia, Africa, Europe, North and South America, and Australia.

The plants from which Nagai isolated ephedrine for the first time was thought to be *Ephedra vulgaris* Rich., but it has since been determined that it was in actuality *Ephedra sinica* Stapf. The latter, together with *Ephedra equisetina* Bunge and *Ephedra distachya* Linn., makes up the commercial form of Ma-huang. It was found that some of the species of ephedra growing wild in northern India also yield *l*-ephedrine in larger amounts than *d*-pseudoephedrine, as does the Chinese Ma-huang. Among the Indian species * are *Ephedra gerardiana* Wall., *Ephedra nebrodensis* Tineo., *Ephedra intermedia*, and *Ephedra pachy-*

* Some of the Indian species give excellent yields. Private communication from Mr. R. L. Brown.

clada. The European and North African varieties of ephedra seem to yield mostly *d*-pseudoephedrine. *Ephedra nevadensis*, which is indigenous to the United States, is void of laevo-ephedrine, but *Ephedra sinica*, which has been cultivated in the United States, yields the usual content of that alkaloid.

The Chinese varieties of Ma-huang are often named after the general area of their occurrence or distribution. Thus *Ephedra equisetine* is generally known under the name of Mupen Ma-huang, and *Ephedra sinica* is known as Tsaopen Ma-huang. Both are marketed chiefly in Peking, which is closest to the mountainous districts of northern China. *Ephedra distachya* is collected in central China and is exported mainly from Tientsin. It is somewhat less desirable because its content of total alkaloids is about 30% lower than that of the other two species.

Ephedra equisetine grows best in sandy soil or clay and is mostly found in the provinces of Chihli and Shansi, and to a somewhat lesser extent in Honan. The plant is a woody shrub of medium height, which is 3–6 ft on the average. The many-branched stem emerges from a long, conical root. The branchlets arise from the stem in whorls and internodes, and they are green and smooth. The leaves are reduced to brownish-purple coriaceous sheaths, which grow around the stems at the nodes to a length of about 0.1 in. The flowers bloom in short male and female spikes. The single, oval seed is contained in a red, fleshy syncarp which looks somewhat like a berry.

Ephedra sinica attains a height of about only 2 ft. Radical branches, somewhat flat and seldom further branched, branch out directly from the base. They are of grayish-green color, slightly striated longitudinally, with the internodes 1–2 in. long. The leaves are reduced to membranous sheaths, up to 0.2 in. long and growing in pairs at the nodes. The flowering spikes are either terminal or at the upper nodes. Two seeds are found in the mature female spike, each having one side flat and one side triangularly convex.

Ephedra distachya attains a height of a little over one foot, with the main stem branching from its upper as well as from its lower part. The branches are rough, greenish-yellow, and the internodes are 1–2 in. long, with the leaves resembling those of *Ephedra sinica*.

According to the *U. S. Dispensatory*: "Ephedra occurs as branched or unbranched, jointed, rounded, and more or less flattened green stems and branches, or as the aboveground portions of the plants with or without the upper part of the woody root attached, fracture brittle, odor aromatic upon crushing, taste aromatic, bitter or astringent." A histological description of the drug will be found in the reference mentioned.

A listing of the various species of Ephedra, their content of total alkaloids in per cent, and the proportion of that of laevo-ephedrine and of dextro-pseudoephedrine will be found in Table 8.2.

TABLE 8.2. ALKALOID CONTENT OF EPHEDRA SPECIES

Species	Total Content, %	% of Total		Reference
		l-Ephedrine	*d*-Pseudo-ephedrine	
equisetine	1.75	90		A
sinica	1.3	90		A
gerardiana	1.7	90		A
intermedia	1.17	90		A
vulgaris	0.73, 0.1		100	B
nebrodensis	2, 3.4	55	100	C
sinica	1–1.6	100		D
alata	0.75–0.88			E
fragilis	0.03		100	F
procera	0.12		100	F
vulgaris helvetica	positive		positive	G
andina	0.38		100	H

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The drug has been extensively investigated not only with regard to the variation of content between the different species, but also with regard to the distribution of alkaloid within the plant, the influence of environment, and the cultivation of the plant.

Very promising and interesting have been the experiments performed by Christensen and Hiner, who cultivated Chinese *Ephedra sinica* in South Dakota.[26] Both the roots and stems were found to be hardy perennials, and the plant was well adaptable to this climate. The following conclusions were reached by these investigators concerning the cultivation of the plant:

Seed should be sown in the spring, just as early as possible, and they should be placed in rows, 30 in. apart and about ½–¾ in. deep. Until germination is well advanced, plenty of water is needed by the seeds. After the second or third growing season, the young plants are transplanted to the field.

It was found that propagation was possible from seeds as well as from runners. Mature plants send out lateral rhizomes, which developed new plants at the tips. After a time the lateral connecting rhizome can be cut. The new plant soon develops roots of its own, and it can finally be transplanted in the following spring. Optimum results were obtained when about 6500 plants were planted per acre.

They also confirmed the fact that Ephedra is essentially a dry-land plant which will not thrive in low, wet surroundings. The fruits (berries) were collected by hand, but, to ensure optimum germination, the seeds were not removed. Production of both seeds and stems increased as the plants matured. Alkaloid content of the stems was related directly to their ages, with the content increasing from year to year.

Harvesting should be undertaken in late September or early October before the first frosts affect the plants. Curing in the field in the sun was found satisfactory, but the process is tedious and may have to be continued indoors.

Tang and Wang [27] have shown that drying of the drug at elevated temperatures appreciably lowers its alkaloid content, and that storing of the drug in moist places is also very injurious, and may eventually destroy its content of alkaloids altogether.

The following method of assay was employed by Christensen and Hiner (*loc. cit.*):

10 grams of finely powdered sample (making allowance for the content of moisture) are placed in a percolator, and are macerated for 5 min with a mixture of ether and chloroform 4:1. After that time 10 cc of ammonia (28%) are added and the mixture is allowed to stand for 15 min. After that time an excess of the solvent is added and maceration is continued overnight. After that time 300–400 cc of the percolate are withdrawn, extracted with dilute sulfuric acid (2%), and the aqueous acid extract is washed with ether which, in turn, is rewashed with dilute sulfuric acid. The solution was then made alkaline with ammonia, extracted with ether, and from then on the determination was continued as described in the *U. S. Pharmacopœia.*

Official in the *U. S. Pharmacopœia* (8th Edition) are ephedrine, as well as ephedrine hydrochloride, sulfate, and ephedrine sulfate tablets, while the *National Formulary* (8th Edition) lists ephedrine hydrochloride tablets, ephedrine spray and compound ephedrine spray, ephedrine

sulfate ampules, capsules, jelly, solution and syrup, and ephedrine sulfate and phenobarbital capsules. Both ephedrine and ephedrine hydrochloride are also official in Great Britain.

MANUFACTURING METHODS

The manufacture of natural ephedrine, although it has been supplanted to a large extent by the manufacture of synthetic ephedrine, is still of some importance, mainly because of the patent situation. A short review of the different synthetic methods of making ephedrine will be given at a different place.

For the manufacture of natural *l*-ephedrine the crude drug is shredded to a mesh size of from 20 to 30. The powdered drug is then charged into the extractors where extraction is undertaken with very dilute hydrochloric acid. Large rectangular vessels are often used for this purpose. They are open on the top to facilitate easy charging and removing of the exhausted drug, and they are usually made of sheetiron.* A dilution of 1:1000 is usually sufficient. Instead of hydrochloric acid a very dilute solution of oxalic, tartaric, or acetic acid can be used. The advantage of using a dilute organic acid is that corrosion of the equipment, including the extractors and pumps, will be greatly diminished. The first two extractions should be undertaken in the cold; the third and fourth extraction can be heated to approximately 50°. The system of countercurrent extraction can be used to some advantage because the final evaporation of the extractions is, by necessity, a long and drawn-out process. Evaporation can be undertaken either in a still under vacuum or in open kettles in the open.

Because of the corrosive action of hot dilute acid solutions the latter method of evaporation is to be preferred because the cheapest kind of open cast-iron equipment can be used. These kettles can be heated through coils or preferably through a jacket. They should be placed in the open where the fumes can do the least damage. A bottom outlet should be provided. In these kettles the extract can be concentrated to approximately ¼ of their volume. Final evaporation and concentration to a thick syrup must be undertaken under vacuum in a still provided with a stirrer.

Concentration must be discontinued before the residue curls into hard lumps. If this were to happen, the removal of the extract from the still would be rather difficult and a considerable loss of alkaloid could also be expected. The thick, viscous syrup is removed from the still while still hot and is transferred to a cast-iron extractor. This extractor

* Private communication from Mr. R. L. Brown.

should be equipped with a stirrer, but mixing by means of live steam has proved quite successful. A glass-lined mixer is to be avoided at this stage because the strongly alkaline liquid would ruin the glass-lining in very short order.

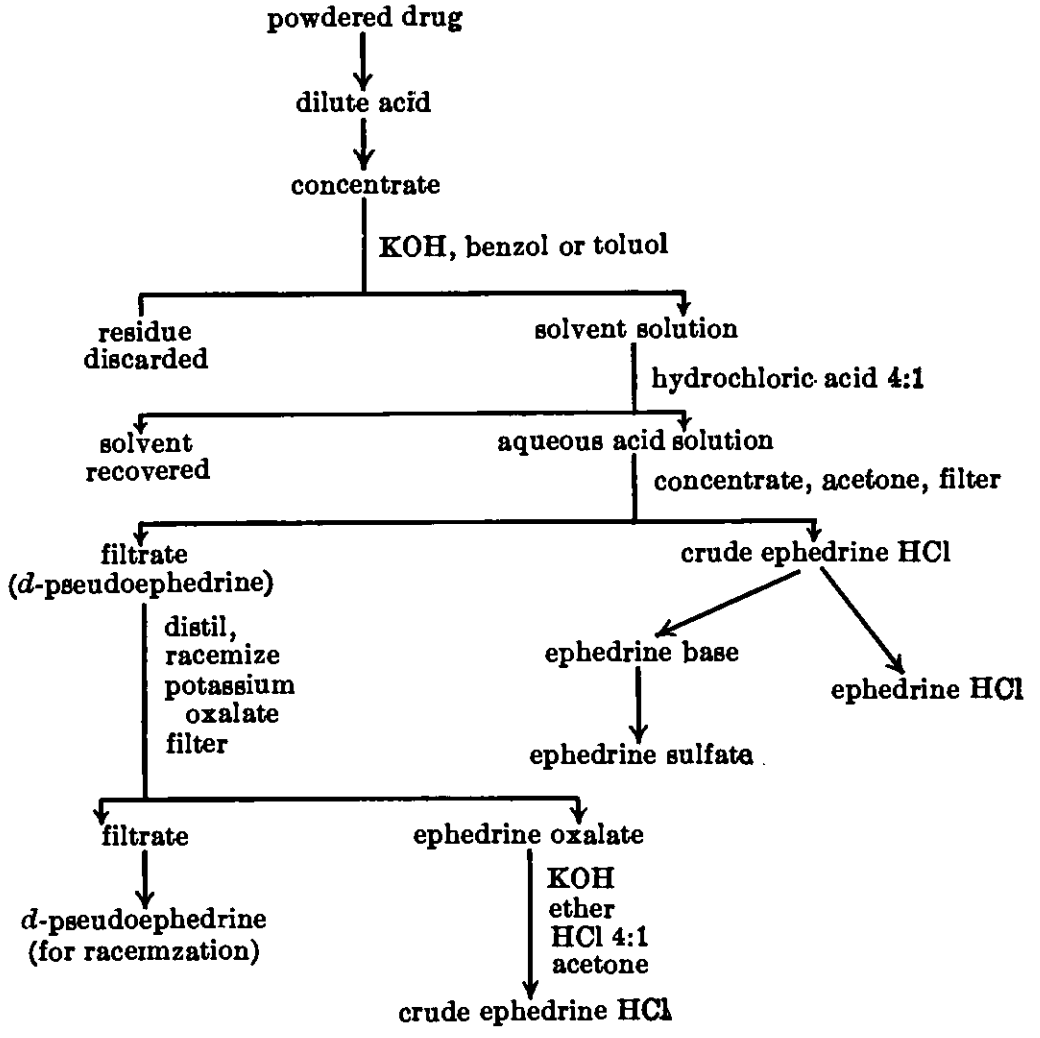
The still-hot extract is mixed with an equal volume of toluol or benzol with the former to be preferred for reasons of health. A large excess of technical caustic soda solution is now added. After thorough mixing and heating, the toluol is allowed to separate for about one hour and is then drawn off from the top by means of a transfer pump and a suitable arrangement of pipes or hard rubber hoses. This solvent extraction is transferred to a second mixing tank which can be glass-lined and should be equipped with a stirrer. The ephedrine and other alkaloids are removed from the solvent by extraction with 10% hydrochloric acid which is allowed to separate for about one hour and is then drawn off and stored. The solvent is returned to the alkaline extract and extraction is continued in the manner described above until all the bases have been removed from the extract. Depending upon the efficiency of the stirring and the time allowed for extraction, approximately 5–8 extractions are usually necessary. It has been found very helpful to heat the mixture during each extraction with live steam if so desired. The disadvantage of using live steam is the eventual accumulation of a large amount of liquid necessitating further additions of alkali. The alkaline extract can be discarded when void of alkaloids. The solvent can be held over for further use or can be recovered.

The combined aqueous extracts are adjusted to a pH of approximately 5.5 to 6 by the addition of sodium hydroxide and are then charged into a glass-lined still and evaporated under vacuum.

This operation should be conducted at a fairly high temperature so as to prevent premature crystallizing out of the hydrochlorides of the mixture of alkaloids. At that point, when definite signs of crystallization of the syrup in the still can be noted, the still-warm extract is blown directly into acetone under continuous stirring. The arrangement described in a previous chapter is most useful for this purpose. A large amount of acetone is necessary to effect a clean separation of *l*-ephedrine from secondary bases and to allow at the same time for a considerable loss of solvent in the course of this operation. The crude ephedrine hydrochloride is separated either by filtration or by centrifuging after having been allowed to crystallize in the cold overnight. The filtercake is washed well with acetone, and the combined filtrates and washings are held for recovery of the pseudoephedrine.

The crude ephedrine hydrochloride is dissolved in a sufficient amount of water to give a 10–15% solution which is then treated with charcoal

and filteraid in the pressure filter as described elsewhere. The resulting solution must be colorless. It is worked up in different ways according to whether the base, the hydrochloride, or the sulfate is desired.



a. For Alkaloid. The solution is cooled and is made alkaline with a large excess of potassium hydroxide. The base is removed by repeated extraction with ethyl ether. The exhausted aqueous phase can be used to make the crude extract alkaline in preparation for extraction with toluol. The ether is carefully separated from any water which might collect after standing overnight and then is distilled off without further washing or drying. The residue is allowed to crystallize and the resulting hemi-hydrate is centrifuged or filtered off and is washed with water once. The resulting hemi-hydrate can either be used as such or it can be transformed into the anhydrous base. To affect this transformation the crystals are returned to the still where they are melted down and dried by heating to approximately 80–90°, bubbling through at the

same time a stream of air which has previously been dried and freed of carbon dioxide by passing over soda lime. A simple test for the presence of moisture can be conducted by dissolving a small sample of the base in light mineral oil. If water is present a cloudy solution will result. The dry ephedrine which should be colorless is allowed to solidify and is then broken into lumps ready for further use.

b. For Hydrochloride. The clear and colorless aqueous solution is concentrated under vacuum to a thick syrup which is then poured into acetone or isopropyl alcohol and allowed to crystallize. The resulting crystals of ephedrine hydrochloride are filtered or centrifuged and are washed with acetone. Since this material may contain more ash than is permitted in the official preparation, it is often preferable that the hydrochloride be made from the base by neutralizing the latter with hydrochloric acid (1:1) and evaporating the resulting solution as described previously. An excess of hydrochloric acid is to be avoided, because the crystals of finished material often include free acid and the pH of the aqueous solution in that case will not meet the official requirements.

c. For Sulfate. The hemi-hydrate, the manufacture of which has been described before, is carefully neutralized with 25% sulfuric acid, and the resulting solution is treated with charcoal in the pressure-filter, evaporated under vacuum, and poured into acetone. If large crystals are desired, the hot syrup is diluted with four times its volume of ethanol or isopropanol, and crystallization is allowed to proceed slowly overnight. Filter or centrifuge, and wash with acetone until colorless.

The acetone motherliquors from the manufacture of crude ephedrine hydrochloride contain *d*-pseudoephedrine, which in the interest of efficient manufacturing should be racemized and the resulting mixture of *d*-pseudoephedrine and *l*-ephedrine resolved. All acetone motherliquors, including washings from the different salts, are combined, and the acetone is removed. It is not suitable for further use because of its content of water. The residue is dissolved in 5% hydrochloric acid, and the resulting solution is boiled for at least 48 hr. During that time the volume of the liquid should be kept constant by the occasional addition of hot water. When the racemization is finished, the solution is practically neutralized and is treated with an excess of a strong solution of neutral potassium oxalate. Allow to crystallize for several days, filter or centrifuge the precipitate, and wash thoroughly with cold water. The precipitate is then suspended in water, and the base is liberated with a large excess of sodium hydroxide, dissolved in ether, and the resulting solution of *l*-ephedrine is processed further as described before. The filtrate contains unchanged *d*-pseudoephedrine oxalate.

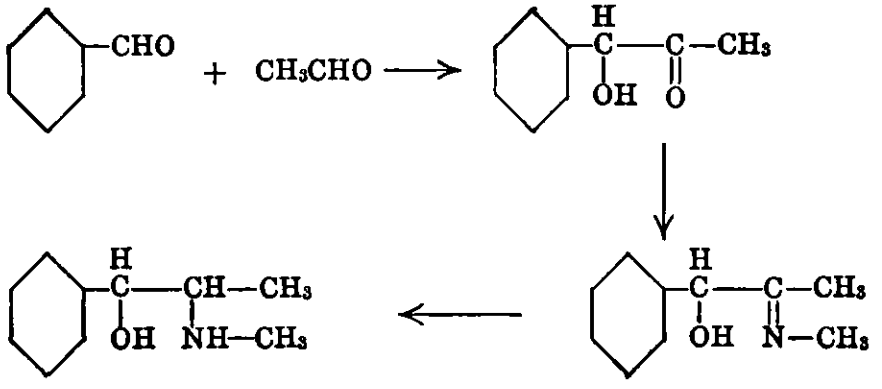
Racemization of ephedrine (*d*-pseudoephedrine) by way of its acetyl derivative has been described previously, but this method has been found to be uneconomical on a large scale. The racemization of ephedrine by means of an alkali-alcoholate has been protected by patent.[28] One tenth to two moles of an alcohol are used per mole of ephedrine, and the boiling point of the alcohol must be below that of the alkaloids (168°/15 mm Hg). An inert hydrocarbon is used as solvent, and the temperature applied is from 135–210°, optimum yield being obtained at 170–190°. With sodium methylate as catalyst, *d*-ephedrine is reported to yield 53% of racemic pseudoephedrine and 42% of racemic ephedrine, but, when sodium methylate is replaced by sodium amylate, 74% of racemic ephedrine is obtained. Racemization of an optically active ephedrine can also be accomplished by heating the base in a non-aqueous medium, such as decaline, with an amide or hydride of an alkali or an alkali-earth metal, such as sodium amide, to 120–210°.[29]

Ghose and Krishna [30] have reported the manufacture, apparently on a commercial scale, or at least easily adaptable thereto, of an Ephedra concentrate, containing 18–20% of ephedrine. They used essentially two methods, a so-called acid method, whereby the Ephedra is extracted with 0.5% sulfuric acid, the extract neutralized with a paste of slaked lime and filtered, the filtrate concentrated to a thick syrup, and the latter triturated with lime and air-dried. In the alkaline method the powdered Ephedra is mixed with 20% of its weight of slaked lime and an equal weight of water. The mixture is dried, extracted with ethanol, which is acidified and concentrated to a thick syrup, which is mixed with water and lime and air-dried.

As indicated previously, most of the commercial ephedrine of today is prepared synthetically, but the most efficient methods have been protected by patent. In addition to the methods mentioned previously, compounds of the type of ephedrine (alpha-alkyl-amino-acylphenones) can be synthesized by reacting a compound of the type of acetophenone or propiophenone with an alkyl-chloramine in the presence of an alkaline reacting substance.[31] Alkamines can also be obtained by heating an aromatic aldehyde with an amino-acid, and both ephedrine and norephedrine have been synthesized in this manner.[32] Stevens *et al.*[33] reduced alpha-bromo-propiophenone with aluminum isopropylate and reacted the bromohydrine thus obtained with methylamine. They obtained racemic pseudoephedrine and racemic pseudo-iso-ephedrine, but no racemic ephedrine.

Most important of all the commercially feasible syntheses is one whereby laevo-ephedrine is produced directly by a method of biosynthesis.[34] Benzaldehyde, when introduced into an actively fermenting

sugar solution in the presence of yeast and the enzyme carboligase, unites with nascent acetaldehyde to form laevo-1-phenyl-propanol-1-one-2,[35] the chemical synthesis of which has not been accomplished yet. This hydroxyketone can be extracted with ether and purified by distillation. It condenses exothermically with methylamine, and the resulting unsaturated compound can easily be reduced by any standard method:



PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

Physiologically, ephedrine is a sympathomimetic amine, and its action in some respects is similar to that of epinephrine or adrenaline, with hydroxysubstituted ephedrines occupying a position intermediate between ephedrine and epinephrine.[36]

Ephedrine causes an increase in the blood sugar, and a general relaxation of the intestinal and bronchial muscles.[37] It has some mydriatic action, for which it was first noted, and it causes a marked rise in blood pressure, this last action being due probably to the anti-enzymatic effect on an epinephrine destroying enzyme.[38] But the most important effects of ephedrine are like those of epinephrine, namely, on the peripheral endings of the sympathetic nerves. The effects of ephedrine, however, are less severe and less pronounced, and they are of longer duration. Upon intravenous injection ephedrine shows a pronounced cardiac action in a decrease of the pressor action of that organ, resulting in eventual heart failure. Concurrent injections of ephedrine and digitalis are advised against because of the resulting upsetting of the rhythm of the heart.[39] Ephedrine causes nervousness and insomnia because of a stimulation of parts of the cerebrum.

Ephedrine can be given by mouth, whereby it retains its pressor and, to a certain extent, vasoconstrictor action. Because of its dilating action on the bronchi, it is used for the relief of bronchial asthma and of hay-fever. Administration prior to paroxysm, but during the prodromal symptoms, is indicated to overcome the lapse of time until its

action becomes effective. Its use as a stimulant in cases of circulatory failure is doubtful,[40] but it is used for the control of angioneurotic edema and of urticaria. Ephedrine has been suggested in the treatment of narcolepsy, as a preventive for serum sickness in the administration of antitoxins,[41] for the treatment of nocturnal enuresis,[42] and of myasthenia gravis.[43]

Dextro-ephedrine and laevo-pseudoephedrine are the least active of its isomers, and much conflicting evidence has been presented concerning the activity of racemic ephedrine,[44] but dextro-pseudoephedrine resembles laevo-ephedrine more than the other isomers.[45] Some attempts have been made to correlate chemical structure and physiological activity by Edkins and Linnell,[46] Hartung *et al.*,[47] Burgmann,[48] and many others.

ANALYTICAL AND TOXICOLOGICAL METHODS

The quantitative determination of ephedrine is described in the *U. S. Pharmacopœia* (8th Edition), in the *National Formulary* (8th Edition) and in the *British Codex* (4th Add.). In addition to the method described previously in this chapter, methods for the estimation of ephedrine have been described by Feng and Read,[49] by Krishna and Ghose,[50] by Moraw,[51] who employed titration, and by Sanchez,[52] who utilized the formation of iodoform. Biological methods have been described by Read and Feng,[53] Githens,[54] and Pittenger.[55]

According to Chen and Kao,[55] 1 cc of a solution of an ephedrine salt (hydrochloride), when mixed with 0.1 cc of a 10% solution of copper sulfate and 1 cc 20% sodium hydroxide, yields a purplish color which can be extracted with ether, whereby the aqueous layer turns blue.

Erdmann's reagent or concentrated sulfuric acid, upon addition to a solution of ephedrine, shows a pink ring on top and a yellow ring below the intersurface. Potassium dichromate in sulfuric acid yields a greenish color which turns brown. There is no reaction with ferric chloride.

A microchemical identification of dextro and of racemic ephedrine are described by Paris,[57] who treats the hydrochlorides with potassium oxalate or sodium vanadate. The resulting crystals can be readily identified.

Fourment and Roques [58] have developed a color test, using a solution of osmium tetroxide and concentrated sodium hydroxide. Ephedrine yields at once an orange precipitate which dissolves in boiling hydrochloric acid with a violet color. After a few minutes' standing, pseudoephedrine yields a yellow precipitate which dissolves in boiling hydrochloric acid with a lemon color.

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CHAPTER IX

THE ALKALOIDS OF ERGOT

CHEMISTRY

Secale carnutum, or ergot, contains a number of alkaloids besides other substances which are not alkaloidal in their nature.

Among the latter are a number of amino acids and simple bases, such as betaine, clavine, tyrosine, histidine, and tryptophane,[1] trimethylamine, choline, and acetylcholine,[2] histamine,[3] ergotic acid,[4] ergothionine,[5] cadaverine, putrescine, and isoamylamine,[6] tyramine,[7] and others.

The ergot alkaloids proper can be grouped into two series of alkaloids, those related to ergotoxine and those related to ergotinine. Ergotoxine, which had been thought to be a single substance, has been shown to be actually a mixture of three closely related bases,[8] but the term *ergotoxine* can still be applied to denote the general characteristics of one series of alkaloids. There can also be distinguished pairs of alkaloids, the members of which are interconvertible and belong to the two series mentioned above.

Ergotinine is the optical antipode of one of the ergotoxine alkaloids, possibly ergocristine. To this first pair, a third alkaloid, pseudoergotinine, is related in a most unusual manner insofar as it can be transformed into either one of the two bases.

The members of the ergotoxine series are laevorotatory and physiologically potent, and they have a definite tendency to crystallize with a solvent included as an additive compound, which at times is quite difficult to remove. The opposite is true of the members of the ergotinine series, which crystallize well in the pure state, are physiologically less active, and are dextrorotatory. It has been observed that the members of each pair often crystallize together as a double compound.

The probable constitution of the ergot alkaloids has only been determined fairly recently. Difficulties encountered in the course of the investigations were due mainly to the fact that hydrolysis of the large ergot alkaloids into their component parts was performed so violently and under such drastic conditions that the fragments which could be identified were not sufficient for identification of the whole molecule. It has also been quite difficult to obtain the ergot alkaloids in a pure state.

It was found that oxidation of "ergotoxine" and ergotinine yields benzoic acid or para-nitrobenzoic acid, and that the bases contain four active and replaceable hydrogen atoms each. Ergotamine and ergotaminine, as well as the two alkaloids mentioned before, do not contain any methoxyl groups, but they all have one methylimino group present. Ergotinine and its optical antipode were shown to be amphoteric, owing to the presence of a weakened carboxyl group and not, as assumed previously, because of a phenolic hydroxyl group.[9] All ergot alkaloids, with the exception of ergometrine and ergometrinine, yield ammonia on boiling with dilute alkali, which, in the case of ergotinine, is derived from the isobutyrylformamide present.[10] Ergine has been observed by Smith and Timmis [11] in the course of mild hydrolysis of the ergot alkaloids. Ergine was found to be the amide of lysergic acid which was obtained by Jacobs and Craig [12] when ergotinine was subjected to stronger hydrolysis.

Inasmuch as ergine is present in all ergot alkaloids, and accounts for about half of the molecule, the difference between the alkaloids must be due to differences in the remaining part of the molecules.

In the course of further investigations it was found that ergotinine (and "ergotoxine"), on hydrolysis, yield lysergic acid and ammonia, dimethylpyruvic acid, and a dipeptide composed of *d*-proline and *l*-phenylalanine.[13] Ergotoxine has been shown to be a mixture of ergocristine, ergocornine, and ergocryptine.[8] These bases can be hydrolyzed as follows: All three bases yield lysergic acid, ammonia, *d*-proline, and isobutylformic acid. In addition, ergocristine yields *l*-phenylalanine, ergocornine yields *l*-valine, and ergocryptine yields *l*-leucine. Ergocristine and ergocristinine had been obtained previously by Stoll and Burckhardt,[14] and had been thought to be distinctly different from "ergotoxine" and ergotinine. Ergocristinine now appears to be identical with the latter.

Ergotamine and ergotaminine yield lysergic acid, ammonia, pyruvic acid, and the dipeptide composed of *d*-proline and *l*-phenylalanine.[15]

Ergosine and ergosinine can be hydrolyzed to lysergic acid, ammonia, pyruvic acid, and a dipeptide composed of *d*-proline and *l*-leucine.[16] Ergosinine, on vacuum distillation, yields *l*-leucyl-*d*-proline-lactame.

Ergometrine and ergometrinine, upon hydrolysis, yield lysergic acid and *d*-beta-aminopropyl alcohol only. These alkaloids are assumed to be the hydroxy-isopropylamides of lysergic acid.[17]

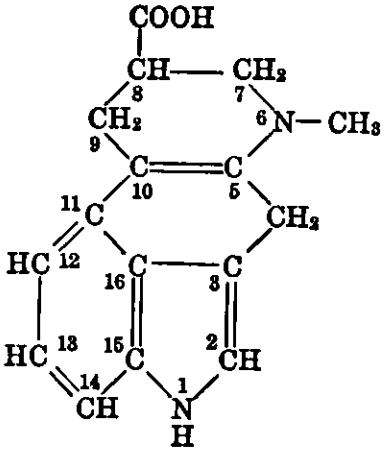
Concerning some of the products of hydrolysis mentioned previously, it has been shown by Jacobs and Craig that the isobutylformic acid and the pyruvic acid are probably derived from alpha-hydroxyvaline and alpha-hydroxyalanine respectively.[18]

A wealth of information has been obtained in reference to ergine and lysergic acid. The following account is intended to be a short survey only; the original literature should be consulted for details.

Ergine, $C_{16}H_{17}ON_3$, first obtained by Smith and Timmis,[11] was crystallized with either water or methanol of crystallization. It was found to be basic, to contain one methylimino group, and to yield ammonia and lysergic acid upon hydrolysis. It can be isomerized to isoergine [19] in the same manner in which the ergotinine-analogue bases can be isomerized.

Lysergic acid, $C_{14}H_{12}N(COOH)NCH_3$, first obtained by Jacobs and Craig,[20] was also found to yield crystalline salts. Different dihydrolysergic acids can be obtained, depending on whether the lysergic acid residue was reduced before or after hydrolysis of the parent base.[21] The acid can be isomerized to isolysergic acid [19] by a reaction which is reversible under different conditions. Lysergic acid and isoergine were found to belong to the ergotoxine-type bases, whereas isolysergic acid and ergine are derived from the ergotinine type series. Lysergic acid has been racemized,[19] and the hydrazide of racemic isolysergic acid has been obtained by Stoll and Hofmann.[22] Under varying conditions this hydrazide could be converted into *d,l*-lysergic or *d,l*-isolysergic acids. Resolution of the latter was effected by means of the nor-*l*-ephedrine salts, but due to racemization during the process of hydrolysis the corresponding optically active lysergic acids were obtained.

Oxidation of ergotinine or lysergic acid with nitric acid yields a tribasic acid, $C_{14}H_9O_8N$,[23] which yields quinoline on distillation with soda lime. Caustic fusion of dihydrolysergic acid gives rise to *l*-methyl-5-amino-naphthalene.[24] On the basis of these and other observations, the following formula for lysergic acid has been proposed by Jacobs and Craig.[25] In the ergotinine series the labile double bond is thought to be at C_9—C_{10}, while in the ergotoxine-like alkaloids the bond is thought to be at C_5—C_{10}.[26]



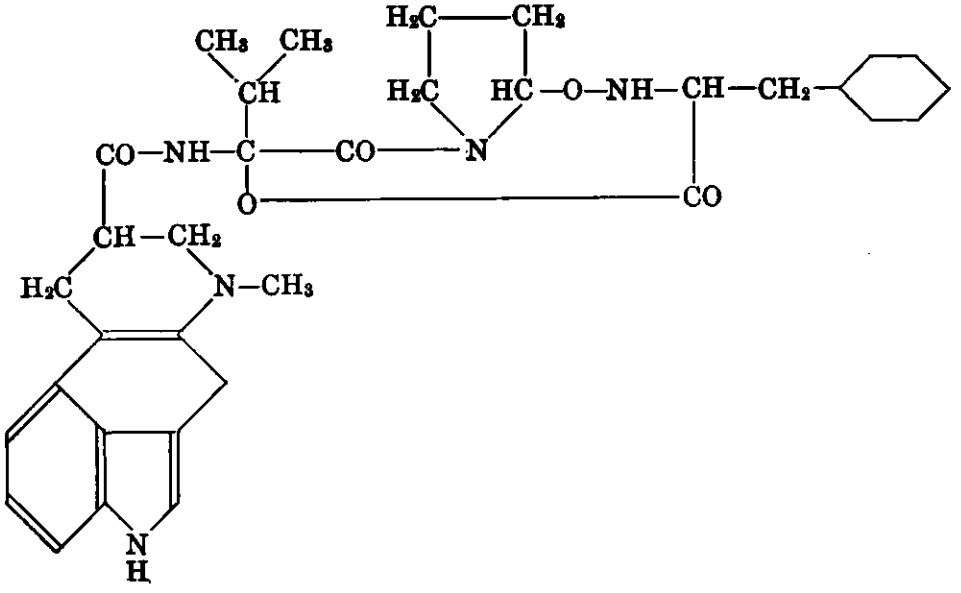
Ergotoxine, which is now known to be a mixture of at least three alkaloids,[8] has been obtained and described by a number of workers.[27] Separation of the three bases was effected by fractional crystallization of the di-(*p*-toluyl)-*l*-tartaric acid salts of "ergotoxine" from alcohol. The mixed "ergotoxine" alkaloids can be obtained from the crude mixture of the total ergot alkaloids by treatment with methanol, which leaves the ergotinine undissolved. Further purification and separation can be effected by means of the slightly soluble sulfates or through the phosphates. The bases can finally be precipitated from their phosphoric acid salts and can be recrystallized from carbon disulfide, toluol, xylol, or benzol, or from dilute acetone (amorphous). They are soluble in ethyl acetate, acetone, chloroform, ethanol, methanol, slightly soluble in ether, and insoluble in petroleum ether.

Ergotinine, $C_{35}H_{39}O_5N_5$ (m.p.: 229°, α_D +365°),[28] crystallizes from dilute acetone. It is converted into ergocristine under the influence of acids.[29]

When a slightly acid aqueous solution of the total ergot alkaloid is made alkaline and extracted with a solvent, the ergotinine is usually removed first. It is sparingly soluble in ethanol, methanol, and acetone, but it is soluble in benzol and chloroform.

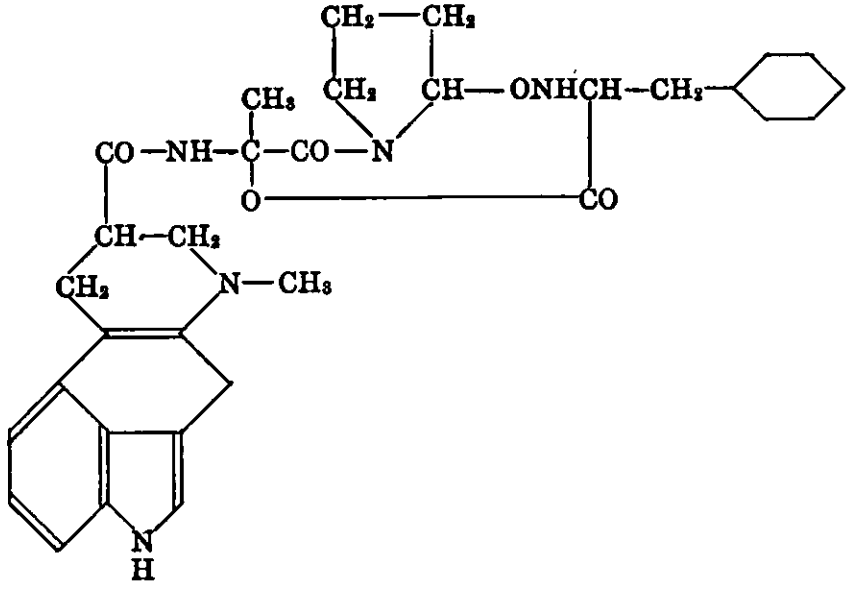
Pseudoergotinine, $C_{35}H_{39}O_5N_5$ (m.p.: 239°), has been converted into ergotinine.[30]

Ergocristine, $C_{35}H_{39}O_5N_5$, has previously been described by Stoll and Burckhardt.[14] The following formula has been suggested for ergocristine and ergotinine.



Ergotamine and ergotaminine, $C_{33}H_{35}O_5N_5$, although isolated by a number of workers,[31] is said to occur in certain species of ergot only.[32]

The separation of the bases is effected similar to the separation of ergocristine and ergotinine. Ergotamine crystallized with aqueous acetone of crystallization (m.p.: 180° (decomp.), α_D −155°, anhydrous base m.p.: 213–214°). Its sulfate, like that of ergocristine, is only sparingly soluble. The base is easily soluble in nitrobenzene and pyridine. It can be purified by crystallization of the phosphate from acetone. Ergotaminine (m.p.: 252° (decomp.), α_D +381°) does not form crystalline salts, similar to ergotinine. It is soluble in chloroform, nitrobenzene, and pyridine, but only sparingly in most other organic solvents. The following formula has been suggested for both alkaloids:



Ergosine and ergosinine, $C_{30}H_{37}O_5N_5$, have been isolated by Smith and Timmis.[33] The two alkaloids are interconvertible.

Ergometrine and ergometrinine, $C_{19}H_{23}O_2N_3$, have also been described by several authors.[34] The former crystallizes in two isomorphic forms (m.p.: 163° and m.p.: 212°), with the latter being the more stable form. The base can be reduced to dihydroergometrine (m.p.: 225–230° (decomp.)). Both alkaloids have been prepared by pseudosynthesis from lysergic acid by Stoll and Hofmann.[22] The latter proved to be *d*-isolysergic-*d*-beta-hydroxy-isopropylamide (m.p.: 196°, α_D +416°), and it could be isomerized to the former, which was then identified as *d*-lysergic-*d*-beta-hydroxy-isopropylamide (m.p.: 159–162°, α_D +91°).

NATURAL SOURCES

According to the *U. S. Pharmacopœia* (8th Edition): "Ergot is the dried sclerotium of *Claviceps purpurea* (Fries) Tulasne (Fam. Hypocreaceae) developed on rye plants. The potency of ergot shall be such

that, when assayed as directed, 1 gram shall be equivalent to not less than 0.5 mg of the U.S.P. Ergotoxine ethanesulfonate Reference Standard. It contains not more than 6% of moisture, and not more than 4% of seeds, fruits, and other foreign organic matter."

Ergot is a fungus which infects many plants in the family Gramineae or Cyperaceae, where it takes the place of the grains or fruits, but it is most frequently found in rye, *Secale cereale*. Ergot-like formations have been found on wild rice, wild rye, oats, wheat, and a number of other plants. The fungus undergoes three distinct changes.

The spores are transmitted to the young ovaries of rye in the late spring or early summer. They germinate, and their hyphae finally envelop and destroy the whole ovary, in whose place is now found a hard, compact pseudoparenchyma. This purplish or violet curved body, which is found instead of the grain, represents the sclerotial or resting stage of the fungus, and it is this material which is the medicinally useful ergot. That ergot, which falls to the ground, germinates in the following spring, and grows small stalks terminated by ascocarps or stromata, which eventually grows the ascospores. These spores are transmitted to the grain by air currents or insects and are ready to start a new cycle.

Ergot, although it resembles grain, has nothing in common with it. It is dark purplish on the outside with an almost white interior. It is made up of large, somewhat oily cells, almost free of starch. A full description of the drug will be found in the *U. S. Pharmacopœia* (8th Edition).

The artificial infection of rye and wheat has been performed successfully with conidiospores, which were obtained from ascospores grown in an artificial medium.[35] Ergot is now produced commercially in Minnesota.[36] It is usually collected prior to harvesting. No relation has been established between the size of the grain and its content of "ergotoxine."[37] Much ergot is imported from Europe.

MANUFACTURING METHODS

The manufacture of the ergot alkaloids on a large scale presents certain difficulties, which are due to a variety of causes. Not only does the alkaloid content of crude ergot vary from species to species, but also different lots from the same species sometimes seem to vary in their content. This variation may be due to differences in the soil, in the climate, or in the time of harvesting, the manner of drying, etc. The second difficulty is due to the fact that most of the industrially successful methods of manufacture are protected by patents. The following

survey is intended to give an over-all picture, describing to the greatest possible extent the processes which have been adapted to large-scale manufacture.

Three products related to ergotamine are obtainable in commerce. They are ergotamine itself, ergotamine tartrate and sensibamine, which is a mixture of ergotamine and ergotaminine. The preparation of all three is described in both the literature [38] and in patents.[39]

It was found that when ergot (2 kg) is mixed with aluminum sulfate (0.2 kg) and water (0.3 kg), and is then extracted with a solvent such as benzol, only fats and other nonalkaloidal impurities are removed. When a sample of this solvent extraction shows only a little residue upon evaporation, the remaining benzol is drained off well, the drug is covered with fresh benzol, and gaseous ammonia is introduced until the whole batch is alkaline. All the alkaloids are now removed by repeated extraction with benzol, the solvent extractions are combined, dried, and concentrated at a low temperature to 50–100 cc. The ergotamine is permitted to crystallize. After it has been filtered, the mother-liquors are evaporated further, and a second crop of ergotamine is obtained by precipitation with petroleum ether. The total crude ergotamine is dried under vacuum, recrystallized from 5–10 times the amount of dry acetone which is diluted with 10% of water after filtration, and is finally recrystallized from 20 times of a mixture of chloroform with 5% of methanol. Ergotamine tartrate is finally produced by mixing the calculated amounts of ergotamine and tartaric acid in 80% methanol.[39]

According to another method,[40] sensibamine is obtained by defatting powdered ergot with petroleum ether, mixing it with wet magnesium oxide, and extracting it with either benzol, chloroform, or ethylene dichloride. The alkaloids are then removed from the solvent by extraction with dilute hydrochloric acid, which in turn is made alkaline again; the alkaloids are re-extracted with a solvent which is concentrated under vacuum after drying. Further purification is effected by dissolving the crude alkaloids in 3% acetic acid, precipitating the sulfates by the addition of sodium sulfate, filtering them, liberating the alkaloid by treatment of the precipitate with sodium bicarbonate, extracting it with chloroform, and finally crystallizing it from that solvent.

Ergotamine can also be purified by adsorbing it from a crude benzol extract unto milk-sugar impregnated with benzol. Ergot fats and ergotaminine are eluted first. The ergotamine is finally purified by crystallization from dilute acetone.[41]

Ergotamine tartrate has also been prepared by extraction of the powdered ergot with liquid ammonia, reducing the volume of the ex-

tract, dissolving it in ether, driving off the last traces of ammonia by warming or by extraction with water, and extracting the alkaloid with 1% tartaric acid solution. After alkalinization with sodium bicarbonate and extraction with ether, ergotamine is crystallized as the tartrate.[42]

Ergometrine has been isolated by Dudley [43] by the following method: 1 kg of defatted powdered ergot was extracted with 1.5 liters of a mixture of absolute alcohol with 5% of methanol and 50 cc of concentrated ammonia. Three liters of the percolate are evaporated to 200 cc under vacuum at a low temperature, and then 300 cc of 0.25 N sulfuric acid are added until a faint acid reaction is apparent. The remaining alcohol is removed under vacuum. After removal of the fat, the liquid is made alkaline with sodium carbonate, extracted with chloroform, and the ergometrine is allowed to crystallize after filtration.

Several patents have been issued protecting the manufacture of ergometrine.[44] The separation from other ergot alkaloids is made possible by the fact that when a crude mixture of the total crude ergot alkaloids is dissolved in a dilute mineral acid, the other alkaloids will crystallize in the form of their salts, but ergometrine will remain in solution, and can be extracted after filtration and alkalinization of the filtrate. The isolation can also be effected by the formation of a salt of ergometrine with an organic acid, such as maleic, oxalic, malonic, or malic acid, by mixing a solvent solution of the alkaloid with a solvent solution of one of these acids.

The manufacture of ergotinine and "ergotoxine" has been the subject of patents both in this country and in Germany.

According to one method, a dilute aqueous slightly acid solution of the total ergot alkaloids is made alkaline with sodium hydroxide and extracted with ether, from which ergotinine is obtained after concentration and crystallization. The aqueous motherliquor is then slightly acidified with lactic acid and extracted with ether, which is then distilled, and the ergotoxine is recrystallized as its phosphate from alcohol. The base is finally liberated and recrystallized as usual. Other acids can be used instead of lactic acid, for instance, acetic, tartaric, hydrochloric, or phosphoric acid. Benzol or trichlorethylene can be used instead of ether.[45]

The extraction of ergot with liquid ammonia or liquid sulfur dioxide has been the subject of other patents,[46] as well as the purification of ergotoxine following that extraction.[47]

Following the extraction with either of the foregoing solvents, ergotoxine can be isolated by precipitating all other bases with a differential precipitant at pH 4, whereby ergotoxine will stay in solution. Some of the compounds mentioned are anthraquinone-2-sulfonic acid or its

sodium salt, beta-naphthylamine-3,6-disulfonic acid, ethyl-mercuric-thiophenyl-*p*-sulfonic acid, the disodium salt of 2-amino-naphthalene-6,8-disulfonic acid, anthraquinone-1-sulfonic acid, or its sodium salt, anthraquinone-1,5-disulfonic acid, and *p*-sulfhydryl-phenyl-sulfonic acid. The solid total alkaloids are treated with a dilute solution (0.1–0.3%) of one of the foregoing substances, the precipitated alkaloids are filtered off, and the ergotoxine is liberated from the motherliquor. The solution is adjusted to pH 2 and is saturated to 50% with sodium chloride and filtered. The filtrate is washed with ether and chloroform. The solution is then adjusted to pH 7.2–7.4 with sodium carbonate or sodium bicarbonate, and is extracted three to four times with chloroform, butyl alcohol, cyclohexanol, methylnaphthalene (with 5–20% of alcohol), but not with ether, benzol, or trichlorethylene. From this solvent extraction the alkaloid is obtained in the usual manner.

According to the same patent, extraction of 500 lb of defatted ergot with liquid sulfur dioxide yields 12 lb of residue. This is dissolved in 5.7 liters of 95% ethanol, poured into 25 gal of distilled water, treated with 114 grams of silver salt, and the mixture is stirred for 3.5 hr. It is then filtered, 35 lb of sodium chloride is added, and the solution is filtered again. After having been made slightly alkaline with sodium bicarbonate, the alkaloid is extracted with a mixture of chloroform and ethanol 4:1, followed by two more extractions with chloroform. An ethereal solution of 10 grams of maleic acid is added to the combined previous extractions, and the alkaloid is separated as its maleic acid salt.

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

Although the alkaloids of ergot show some slight differences in their general physiological actions, they all cause the pregnant uterus to contract. Injection of fluid extract of ergot is followed by a reduction in the rate of the pulse and a rise in blood pressure. If epinephrine is administered following a large dose of ergot, the blood pressure diminishes contrary to expectations. The ergot alkaloids, although rather slow in their action, are circulatory stimulants. They have been used to relieve local passive congestions, such as occur in diarrhea, spinal or cerebral congestions, chronic vasomotor conditions, internal hemorrhages, or delirium tremens. For their sedative-like effect they have been used in asthma, hysteria, and in cures for habit-forming drugs.[48]

The sympathicolytic and oxytocic action of the ergot alkaloids of the "ergotoxine" series is already shown in traces by the ergine part of the molecule.[49] Iso-ergine, which is obtained from this series of alkaloid, seems to be slightly more active than ergine. The alkaloids of the

ergotinine series show almost no physiological activity.[50] Ergometrine has been shown by Brown and Dale [51] to be less toxic than ergotoxine, to cause less pressor action, to cause cyanosis of the cock's comb but with fewer ill side effects, and to be followed by central excitation and general sympathetic stimulation but very little paralyzing actions. It causes the uterus to contract rhythmically. It is relatively quick acting, even when given orally.[52] Stoll has pointed out that the optical antipode of ergometrine shows little physiological activity.[53]

"Ergotoxine" and the alkaloids of its series show a definite pressor action, cause the uterus to contract, and in larger doses cause gangrene in the cock's comb. Ergotamine seems to be less toxic than ergotoxine. Ergosine resembles the latter, but is somewhat weaker in its action.[54] Excellent reviews of the pharmacological properties of the ergot alkaloids have been published by Barger [55] and by Nelson and Calvery.[56]

ANALYTICAL METHODS

Numerous chemical and physiological methods have been devised for the quantitative estimation of the ergot alkaloids. The chemical methods may be colorimetric or photometric, or may consist of a method of separating and titrating the alkaloids. These methods have the drawback that the physiologically active alkaloids are determined together with the inactive ones. References are given to some of the methods published.[57]

Physiological methods may utilize the antagonistic action of epinephrine and the ergot alkaloids on strips of isolated rabbit uterus, or the darkening of the cock's comb.[58] Special methods for the estimation of ergometrine have been devised.[59]

Most of the ergot alkaloids give similar color reactions: a deep blue with dimethylamino benzaldehyde, a purplish blue in glacial acetic acid with a trace of ferric chloride and concentrated sulfuric acid, a blue color with glyoxylic and sulfuric acid and a yellowish color with nitric acid and sodium nitrite.

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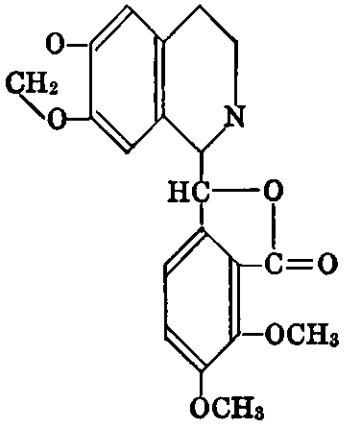
CHAPTER X

HYDRASTINE AND BERBERINE

CHEMISTRY

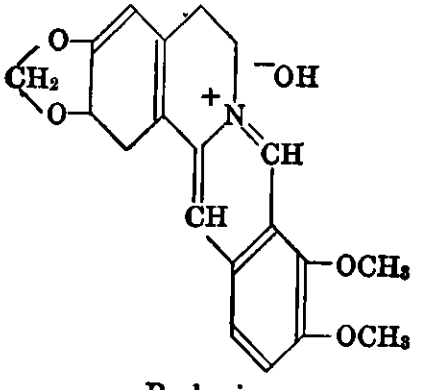
Hydrastine and berberine shall both be discussed in this chapter, mainly because in the usual manner of manufacture they are both obtained from the same crude drug.

Hydrastine is the better known alkaloid occurring in *Hydrastis canadensis*, although it is surpassed in quantity by berberine even there.



Hydrastine

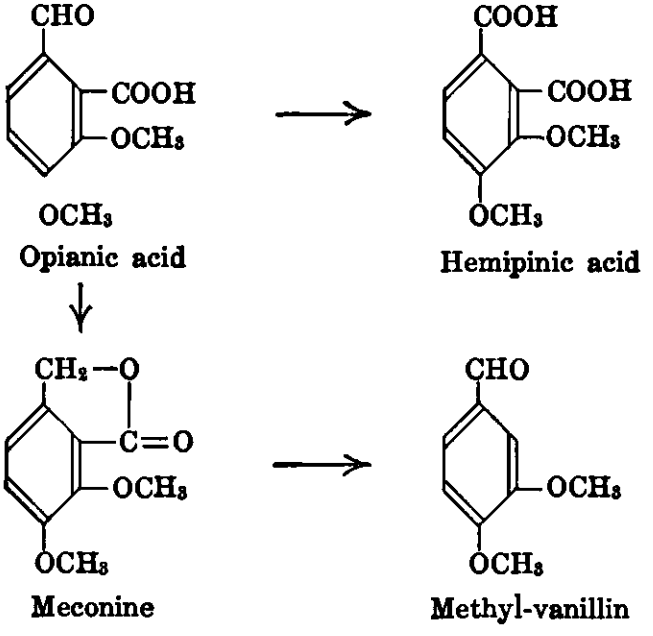
Berberine and berberine-type alkaloids occur in *Hydrastis canadensis* as well as in a large variety of plants.



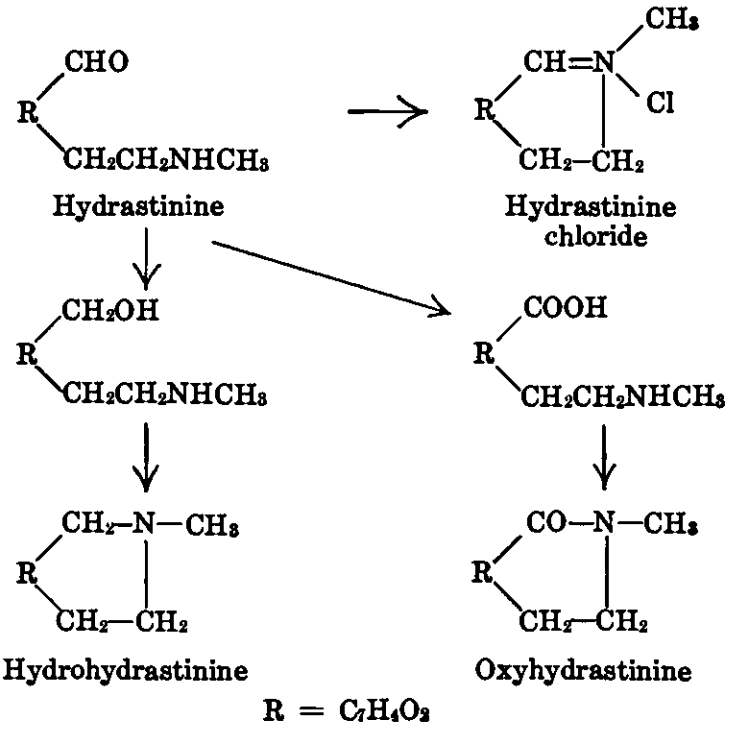
Berberine
(ammonium form)

Hydrastine was found to contain one methylenedioxy group and two methoxyl groups,[1] and to behave as a tertiary base with alkyl iodides. On treatment with dilute nitric acid, opianic acid ($C_{10}H_{10}O_5$) and hydrastinine ($C_{11}H_{13}O_3N$) are obtained.[2] Although one of the products of hydrolysis contains an aldehyde group, hydrastine itself contains none.

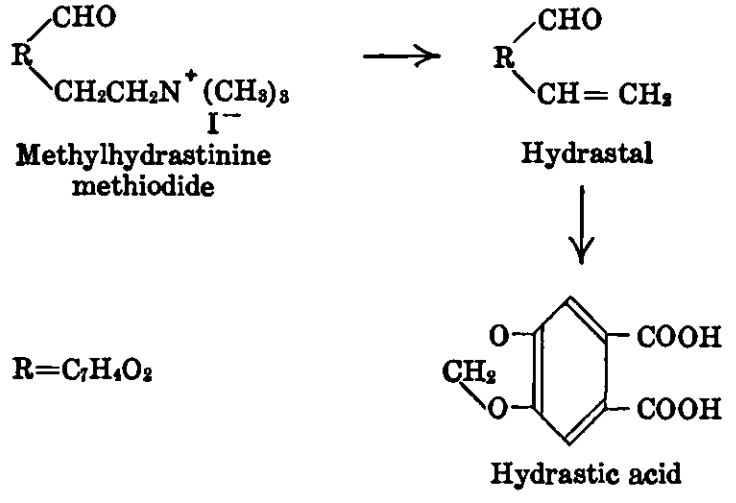
Opianic acid yields hemipinic acid ($C_{10}H_{10}O_6$) upon oxidation. Reduction gives rise to meconine ($C_{10}H_{10}O_4$), which on treatment with soda lime gives methyl-vanillin ($C_9H_{10}O_3$), which can be converted into vanillin ($C_8H_8O_3$) by boiling with hydrochloric acid.



Hydrastinine, $C_{11}H_{13}O_3N$, was found to form an oxime with hydroxylamine (m.p.: 145–146°), to form an acetyl and a benzoyl derivative with acetic anhydride and benzoylchloride respectively, and to contain one methyl group linked to nitrogen. On treatment with sodium or potassium hydroxide, a mixture of oxyhydrastinine, $C_{11}H_{11}O_3N$ (m.p.: 97–98°), and hydrohydrastinine, $C_{11}H_{13}O_2N$ (m.p.: 66°).[3] This reaction seems to involve the formation of an alcohol and an acid from the corresponding aromatic aldehyde.[4]

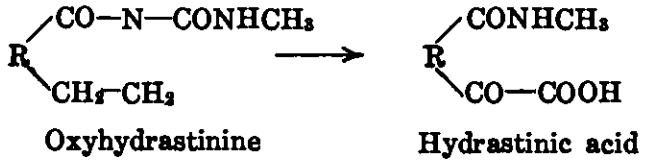


Exhaustive methylation of hydrastinine with methyl iodide gives a mixture of methylhydrastinine methiodide and hydrastinine hydriodide, the first compound yielding the aldehyde hydrastal ($C_{10}H_8O_2$) on distillation with alkali. Hydrastal yields hydrastic acid, $C_9H_6O_6$ (m.p.: 175°), on oxidation.



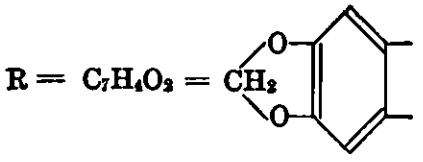
The constitution of hydrastic acid was established when it was found to be a dibasic acid, forming an anhydride, and yielding protocatechuic acid and catechol on fusing with potassium hydroxide. The action of water on the reaction product of hydrastic acid and phosphorous pentachloride produces nor-meta-hemipinic acid.[5] Hydrastic acid was also

obtained by subjecting oxyhydrastinine to oxidation with alkaline potassium permanganate to give hydrastinic acid, $C_{11}H_9O_6N$ (m.p.: 164°), which in turn yields hydrastic acid methyl imide $C_{10}H_7O_4N$ (m.p.: 228°) on oxidation with dilute nitric acid. Hydrolysis with potassium hydroxide yields hydrastic acid.

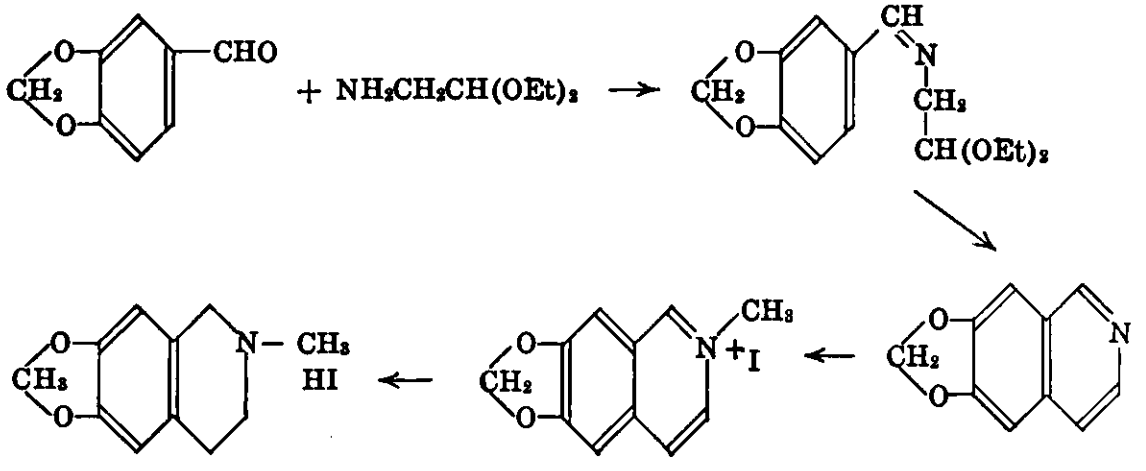


Oxyhydrastinine — Hydrastinic acid

Hydrastic acid — Hydrastic acid methylimide

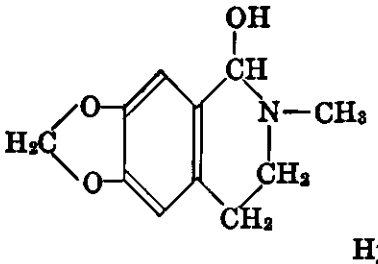


The formula of hydrastinine was deduced from the reactions described previously, and its constitution was first confirmed by Fritsch [6] by synthesis of the base. Piperonal was condensed with aminoacetal to form piperonylideneaminoacetal, which on treatment with sulfuric acid undergoes ring-closure to form methylenedioxy-isoquinoline. This is reduced through the methiodide to hydrohydrastinine hydriodide, which is oxidized to hydrastinine.[6a]



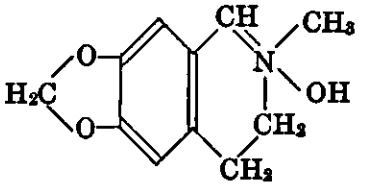
Hydrohydrastinine hydriodide

Because of adsorption spectrum data obtained for hydrastinine, this compound is thought by Dobbie and Tinkler [7] and by Steiner [8] to exist in two forms, the first of which is present in nonpolarized solvents, whereas the second is found in water or alcohol.



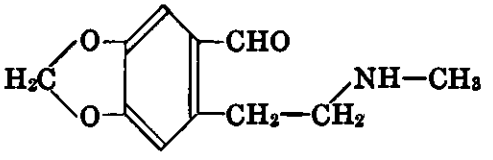
(Carbinol form)

Hydrastinine



(Ammonium form)

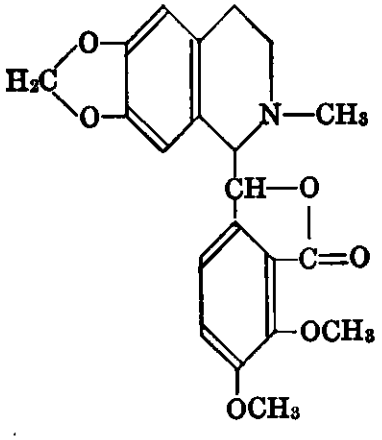
A third configuration of hydrastinine is possible.



(Imino-aldehyde form)

Other syntheses of hydrastinine have been described by Freund and Will,[9] Pyman and Remfry,[10] Decker,[11] and in a number of patents.[12] The commercial methods of synthesizing hydrastinine will be described later.

The following formula for hydrastine itself has been suggested by Freund and Rosenberg,[13] and is based on Roser's formula for narcotine.[14]



Inasmuch as there are two centers of asymmetry, two racemic forms of hydrastine are theoretically possible. One of them was obtained by

Hope and Robinson,[16] who obtained *d,l*-nitrohydrastine from nitromeconin and hydrastinine in boiling alcohol. An optically inactive hydrastine (m.p.: 137°) was obtained by way of the hydrazine derivative. Upon further examination by Hope *et al.*[15] this substance was shown to be a mixture of isomers, and a complete separation could be effected in the amino-phase of the reduction of the nitro-compound. Finally obtained were *d,l*-hydrastine-a (m.p.: 137°) and *d,l*-hydrastine-b (m.p.: 151°). It was also demonstrated by Marshall, Pyman, and Robinson [16] that natural hydrastine (α_D −68.3°) can be partially converted into an isomer of different rotation (α_D −163°) by the action of methanolic potassium hydroxide.

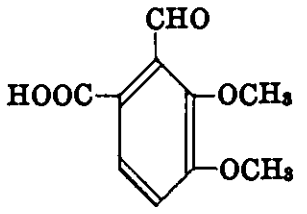
By comparison with natural narcotine (*l*-alpha-narcotine) natural hydrastine differs in its stereochemical configuration from that compound and is therefore called *l*-beta-hydrastine. The new isomer with α_D −163° can then be called *l*-alpha-hydrastine. Again drawing an analogy to narcotine, in the synthesis of which alpha-gnoscopine, but not beta-gnoscopine, can be resolved into dextro- and laevo-narcotine, it is now clear that the total synthesis of natural hydrastine will depend upon the conversion of hydrastine-b into *l*-alpha-hydrastine, which can be isomerized to *l*-beta-hydrastine by boiling with methanolic potassium hydroxide.

Hydrastine crystallizes in rhombic prisms from alcohol, ethyl acetate, or acetone. It is almost insoluble in water, soluble in chloroform (7%) and benzene, slightly soluble in cold alcohol (0.6%) or ether (0.6%). Its optical rotation in chloroform is α_D −67.8°, that in dry alcohol is α_D −49.8°,[17] and in 50% alcohol it is α_D +115°.[18] The rotation of the hydrochloride in dilute hydrochloric acid is α_D +127.3°. The salts of hydrastine are extremely hygroscopic and usually do not crystallize well, with the exception of the picrate, the acid oxalate, and the acid tartrate. The last two mentioned can be crystallized from hot water.

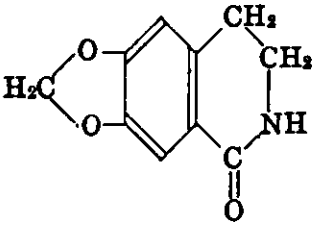
Hydrastinine is optically inactive. It is slightly soluble in water, but dissolves in alcohol and water-immiscible solvents. Its salts crystallize well.

Berberine, $C_{20}H_{19}O_5N$, upon oxidation with warm, alkaline potassium permanganate, was found to yield a variety of oxidation products, all of which were helpful in elucidating the structure of berberine. The more important products are the following:

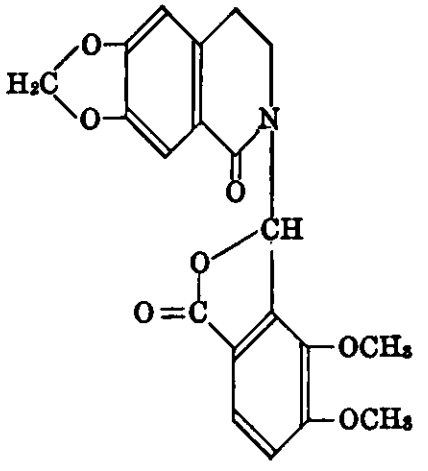
Berberal, $C_{20}H_{17}O_7N$ (m.p.: 150°), which can be hydrolyzed to pseudo-opianic acid and to aminoethylpiperonylic anhydride. The latter can be considered to be nor-oxyhydrastinine, and oxyhydrastinine was prepared from it by Perkin.[19] Pseudo-opianic acid and aminoethylpiperonylic anhydride can be recombined to form berberal.



Pseudo-opianic acid

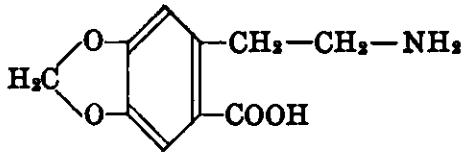


Aminoethylpiperonylic anhydride

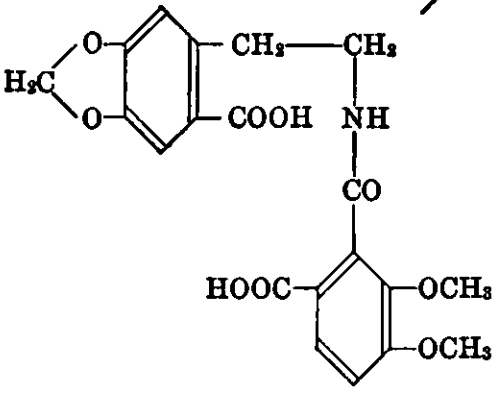


Berberal

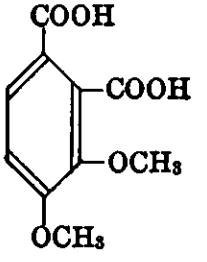
Berberilic acid, $C_{20}H_{19}O_9N$ (m.p.: 181°), was found to be dibasic, to form a dimethyl ester (m.p.: 173°), and to form the anhydride when heated to about 180° (m.p.: 236°). The latter substance is soluble in dilute carbonate solutions, forming berberilates. Hot, dilute sulfuric acid hydrolyzes berberilic acid to hemipinic acid and to α-aminoethylpiperonylic acid (m.p.: 182°).



ω-Amino-ethylpiperonylic acid

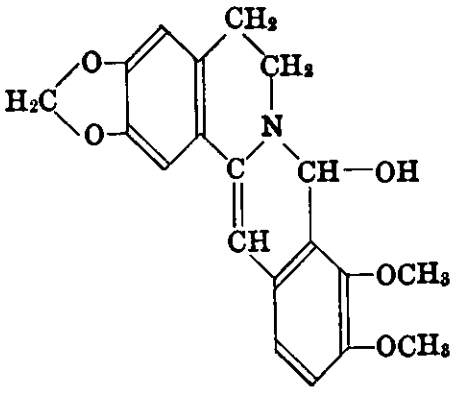


Berberilic acid

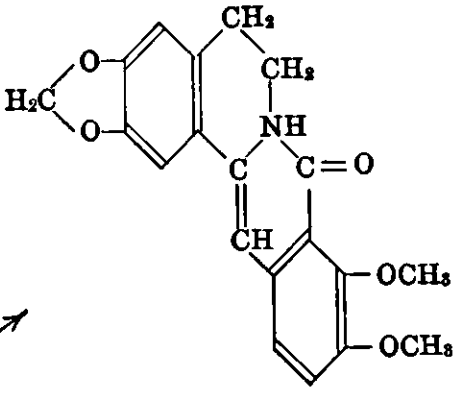


Hemipinic acid

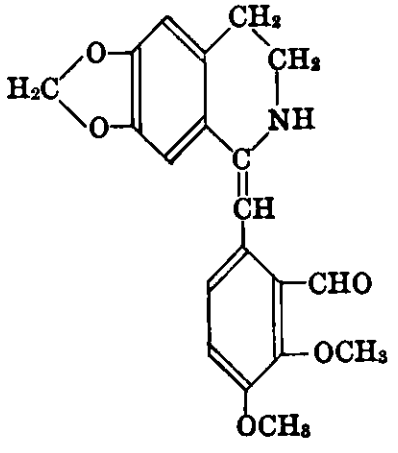
Oxyberberine, $C_{20}H_{17}O_5N$ (m.p.: 200°), besides being an oxidation product of berberine, is also formed together with dihydroberberine, by the action of concentrated sodium hydroxide on two isomers of berberine, the aldehyde form (berberinal) and the carbinol form of berberine.



(Carbinol form)

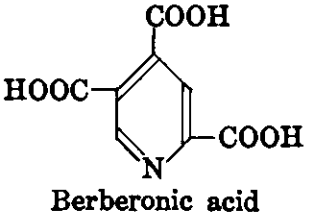


Oxyberberine



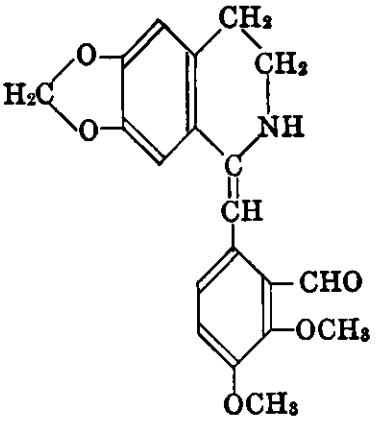
Berberinal

Oxidation of berberine with nitric acid results in the formation of berberonic acid.



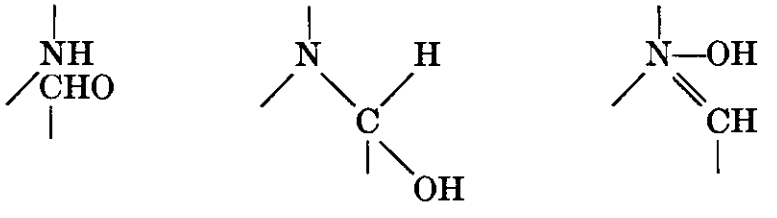
Berberonic acid

Ordinary berberine is thought to be the ammonium form of the alkaloid. It was shown by Gadamer [20] that berberine sulfate, on treatment with the exact amount of barium hydroxide, yields a strongly alkaline, brownish-red solution of berberinium hydroxide. The latter, on treatment with an excess of sodium hydroxide, yields an ether-soluble form of berberine, thought to be the aldehydic form berberinal. This compound has not been isolated as such but only in form of its oxime (m.p.: 165°).



Berberinal behaves like an aromatic aldehyde, giving rise to oxyberberine and dihydroberberine on treatment with strong sodium hydroxide.

Evidence for the existence of berberine in three isomeric states has been given by Tinkler,[21] who found that the ultraviolet adsorption spectrum for ordinary berberine and its salts was alike, but different from the adsorption spectrum of berberinal and dihydroberberine, which again showed some resemblance. Berberine base is probably the carbinol form (berberinol). In solution, salts are formed from the berberinium hydroxide by replacement of the hydroxyl group and the elimination of water. Formulae for berberinium hydroxide and berberinal have been given previously (berberinol (berberine base) is synonymous with berberinal). Possible structures are as follows:



Imino-aldehyde Pseudobase Quaternary base

The structure of berberine has been confirmed by several syntheses. Perkin, Ray, and Robinson [22] heated beta-piperonylethyl-meconinamide with phosphoryl chloride and reduced the reaction product with zinc dust and glacial acetic acid to oxyberberine. Inasmuch as oxyberberine can be reduced further to tetrahydroberberine [23] and the latter oxidized to berberine, synthesis of that alkaloid has been effected.

Canadine, $C_{20}H_{21}O_4N$, the third alkaloid found in *Hydrastis canadensis*, was found by Gadamer [20] to be laevo-tetrahydroberberine, and it was obtained by fractional crystallization of tetrahydroberberine-bromocamphorsulfonate.

Berberine crystallizes with 5.5 moles of water of crystallization from

water or dilute alcohol. When dried at 100°, the crystalline needles retain 2.5 H_2O. They darken at 110° and decompose at 160°. When crystallized from solvents, berberine can contain either chloroform or acetone of crystallization. Berberine is slightly soluble in cold water (25%) and in cold alcohol (1%), but is quite soluble in the hot liquids. The salts crystallize well and are sparingly soluble in cold water.

Canadine was first isolated by Schmidt and Wilhelm who prepared it from crude hydrastine by way of the nitrate. The base crystallizes in needles (m.p.: 132.5°), insoluble in water, easily soluble in most solvents, but very little soluble in petroleum ether. The alcoholic solution is neutral. Varying optical rotations have been reported, α_D −299° in chloroform and α_D −432° in carbon disulfide. The hydrochloride and the nitrate are crystalline, slightly soluble in water, and laevorotatory.

NATURAL SOURCES

Of the several drugs to be considered for the manufacture of hydrastine and berberine, only *Hydrastis canadensis* is official at this time. According to the *National Formulary* (8th Edition), "hydrastis consists of the dried rhizome and roots of *Hydrastis canadensis* Linné (Fam. Ranunculaceae), commonly known as golden seal."

The plant used to occur abundantly in the rich woodlands of North America east of the Mississippi, but wild-growing specimens have become progressively rare, so much so that most of the supply is dependent upon cultivation of the drug. Extensive cultivation can be found in the Pacific Northwest, the Skagit Valley in Washington, in Swain County in North Carolina, also in West Virginia, Kentucky, Indiana, and Ohio. In some areas in these last-named states a supply of the plant is growing wild in virgin forests.

The plant is a small, perennial herb, from 6 to about 15 in. high. The underground portion consists of a horizontal, thick, fleshy rhizome with a fairly large number of long, slender roots. Owing to the presence of berberine, the rhizome is of a deep yellow color internally. From the rhizome arises an erect simple stem, carrying, as a rule, only two unequal leaves, one at the top and the other slightly below, giving the stem a bifurcate appearance. The leaves are pubescent, five to seven-lobed, pointed and unevenly serrate. A single flower appears in April or May on a peduncle, arising from the base of the upper leaf. It has a greenish-white calyx but no corolla, and disappears soon after it has opened. The fruit is a crimson compound berry, looking somewhat like a raspberry, but being nonedible. The fruit contains several fleshy carpels, but usually one, rarely two, seeds. Only one other species

of Hydrastis seems to be known, *Hydrastis jezoensis*, which grows in northern Japan.

As indicated before, most of the commercial supplies of hydrastis are now obtained by cultivation of the plant. Extensive studies have been undertaken in connection with this problem.[24] It was shown that propagation of the plant was possible either from seeds or from cuttings of the rhizome, but that good shade and drainage were absolutely essential. Artificial shade or the natural shade of the woodlands is equally effective, whereas sloping hillsides provide the correct amount of drainage.

The seeds are sown in the fall in seed beds and are covered with a fairly thick layer of fine leaf mold. During the winter they are protected with burlap bags. In the spring when the seedlings have obtained a height of about 2 in., they are transplanted into cold frames or pots for a short time before being transplanted finally in well-fertilized soil in the open, containing a good amount of leaf mold.

When the plants are grown from seeds, the rhizomes can be collected after about five years. When the plants are grown from rhizome cuttings, they can be collected after three or four years.

The rhizomes and roots are collected in the fall after the part of the plant above the ground has withered. They are washed clean and are dried in the shade, protected from rain and sun. They are packed loose into burlap bags and should be kept dry to prevent molding.

According to the *National Formulary* (8th Edition), "Hydrastis contains not more than four per cent of foreign organic matter, and yields not more than three per cent of acid-insoluble ash, and not less than 2.5 per cent of the anhydrous ether-soluble alkaloids of Hydrastis.

"Rhizome subcylindrical, flexous, from 1 to 5 cm. in length and from 2 to 10 mm. in thickness; more or less annulate and wrinkled longitudinally; brown to dusky yellowish orange; marked by numerous stem-scars or occasional stem or leaf bases and numerous roots, frequently broken, leaving circular yellowish-brown to yellow scars or short protuberances; fracture short and waxy. Roots numerous, filiform, up to 35 cm. in length and one mm. in diameter; curved, twisted, and matted together or broken; fracture short and brittle; internally roots and rhizomes weak yellowish orange to moderate greenish yellow."

The crude drug has been extensively investigated regarding its morphology, identification, and assay, by Holm,[25] Rosenthaler,[26] Gillis and Langenhan,[27] Markwell,[28] and Blague and Maheu.[29]

Roots and rhizomes containing berberine are often used to adulterate the crude drug. The following species have been reported: *Jeffersonia diphylla, Poeonia officinalis, Coptis teeta,* and *Xanthorhiza simplicissima,*

also members of the *Serpentaria*, *Cypripeolium* and *Aristolochia* species.

Berberis, no longer officially recognized, consists of the dried rhizome and roots of species of the section Mahonia (Nuttall) De Candolle of the genus *Berberis* Linné (Fam. Berberidaceae). The plants, shrubs, or trees are widely distributed in the temperate regions and in the mountains of the tropics. In the United States, *Berberis aquifolium* and *Berberis nervosa* (Pursh) are found in the Pacific Northwest, from California to British Columbia. These plants belong to the section Mahonia, which alone was recognized in the *National Formulary* and which also includes *Berberis fremontii* Torr., *Berberis repens* Lindl., and *Berberis fascicularis* Lindl. Other members of this genus are found in China (*Berberis fortunei* Lindl.), in India (*Berberis nepalensis*), and in Mexico (*Berberis chococo* Schlect.). All the above-mentioned plants are tall shrubs or small trees, with evergreen, bright, shining pinnate leaves, smooth stems with yellow wood, yellowish-green flowers which appear in the spring, and fruits in the form of pulpy, acidic berries.

The section Euberberis of the genus *Berberis* contains mostly deciduous shrubs, found in Asia, Europe, and North America. Included in this section are *Berberis vulgaris* L., indigenous to Europe but found abundantly in New England, *Berberis canadensis*, *Berberis aristata*, *Berberis lycium* Royle and others. These plants can be distinguished by their long shoots bearing three-parted spines, the undivided foliage, and the yellowish flowers.

The *National Formulary* describes Berberis as follows: "Cylindrical, more or less knotty, strongly branched, usually cut into pieces of varying lengths and up to 45 mm. in diameter; usually splitting somewhat on drying; externally light yellowish brown to pale olive, longitudinally wrinkled, and short-scaly; fracture hard and tough; bark one mm. in thickness, easily separable into layers; wood light yellow to weak greenish yellow, the color more pronounced upon wetting, distinctly radiate, and showing rings of growth; pith or rhizome small, sometimes excentric. It colors the saliva yellow when chewed.

"Berberis contains not more than five per cent of attached overground stems and not more than two per cent of other foreign organic matter, and yields not more than two per cent of acid-insoluble ash. Reject pieces of the rhizome or root over 45 mm. in diameter or with the bark removed." The crude drug is often adulterated with the wood of the stem, which, although it contains berberine, does so to a much smaller extent.

Hydrastis canadensis is official in the *National Formulary* (8th Edition), which also lists hydrastine hydrochloride, hydrastis extract powdered, hydrastis fluid extract, and tincture of hydrastis.

MANUFACTURING METHODS

Methods of Extraction

In this section, the following processes, all of which are commercially feasible, will be described:

(a) Separate extraction of hydrastine and berberine from golden seal root.

(b) Extraction of the total alkaloids from golden seal root and their separation.

(c) Extraction of berberine from berberis.

(d) Extraction of hydrastine from golden seal leaves.

Process (a) is by far the most desirable one, giving quantitative yields of berberine and hydrastine in practically pure condition. Process (b) is somewhat faster than process (a), but the initial saving in time is offset by the fact that the alkaloids are not obtained in as pure a state as in the first process. The need for process (c) for the manufacture of berberine alone is self-evident. Process (d) has been used during the war with some success.

Separate Extraction of Hydrastine and Berberine from Golden Seal Root

This process is an adaption of the method described by Elsa Schmidt.[30] Variations are the elimination of ammonia in the first stage of extraction, and the possibility of a different method of extracting the berberine in the second stage.

Golden seal root is powdered (20–40 mesh) in the usual manner and filled into extractors, which must be equipped with a jacket and should have, if possible, a short reflux condenser. The drug is now covered with toluol, heat is applied at the jacket, and circulation of the solvent is started and is continued until the toluol is near boiling.

During this procedure, the percolator should be covered tightly and an air release pipe should be attached in such a way as to lead all fumes to the outside of the building. A short reflux condenser is desirable to keep the loss of solvent to a minimum, but, in any case, good ventilation for the workers must be provided. Benzol has been used instead of toluol, but it has been found undesirable not only because of its health hazard, but also because of its lower solvent power for hydrastine.

When the toluol has reached near boiling, the hot solvent is pumped into a mixing tank instead of being returned at the top of the extractor. This can be done easily by installing a pipe line from the circulating pump to the mixing tank. The golden seal should be drained well for

maximum efficiency. The toluol is now extracted with a cold, dilute solution of sulfuric acid (2%), and the mixture is stirred gently for a short while to ensure removal of all the hydrastine from the toluol.* After allowing sufficient time for separation, about one half hour, the aqueous acid phase is separated as closely as possible. The toluol is now washed with a small amount of dilute ammonia (1%) to remove any excess acid. The ammonia is separated and set aside, the toluol is returned to the extractor, and heating and circulation are started as described before.

This cycle of extracting the hydrastine from the drug with toluol and removing it from the latter with dilute acid is repeated about five to seven times, until a sample of the aqueous acid solution shows only a little hydrastine when made alkaline with ammonia. This phase of the process has been found effective in individual batches only. Countercurrent extraction with hot toluol was not effective because of the limited solubility of hydrastine in toluol.

When all the hydrastine has been removed from the golden seal, the latter can be made ready for the extraction of berberine. The toluol can be put aside and can be recovered if desired. The acid solution of hydrastine and the ammoniacal washings of the toluol are worked up for the recovery of hydrastine as will be described later.

The berberine can be extracted either with water or with alcohol. If the latter procedure is adopted, it is not necessary to remove all the toluol from the golden seal. If extraction of the berberine is to be undertaken with water, it is best to remove the crude damp drug from the extractors, spread it out on concrete floors in a warm, well-ventilated room until all the toluol has evaporated, and then to charge the drug into wooden extractors. When extracting with acidified water, it is advisable to avoid contact of the liquid with metals. Details of this method will be described later.

Extraction of Berberine by Means of Alcohol

The crude drug, still slightly damp with toluol, is covered with methanol, which is then heated and circulated as described elsewhere. This phase of extraction can be undertaken in a system of countercurrent extraction. The combined alcoholic extracts are evaporated under vacuum to a thick syrup. This syrup, besides containing all the berberine, may also contain traces of hydrastine. The last of this alkaloid can be removed from the residue of the distillation by refluxing the latter for one hour with five to ten times its volume of toluol. The toluol,

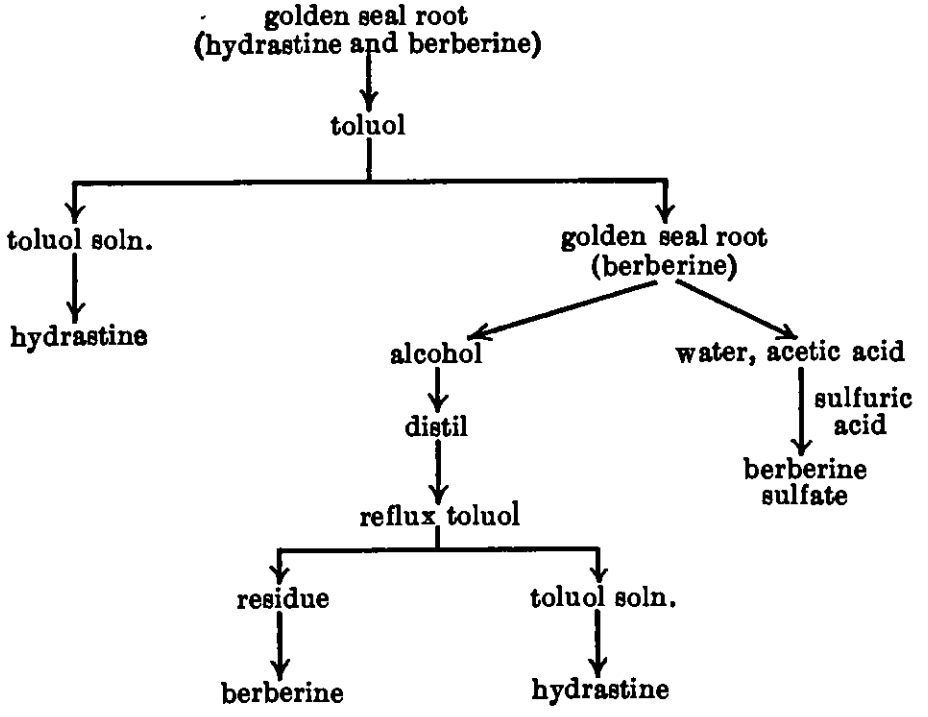
* Vigorous stirring produces emulsions. Private communication from Mr. R. L. Brown.

after separation, will yield a small amount of hydrastine which can be separated by extraction with dilute sulfuric acid as described before. The toluene can then be set aside for recovery. The berberine can be removed from the syrupy distillation residue by mixing the latter with warm, dilute acetic acid (0.5–1%), removing the insoluble material either by filtration with filteraid or by decantation, and crystallizing berberine hydrochloride from the clear liquor by the addition of an excess of concentrated hydrochloric acid. The crude berberine hydrochloride can be filtered off and the motherliquor can be discarded.

Inasmuch as the separation of the aqueous acid phase from the gummy impurities mentioned previously may be cumbersome at times, another method has been devised for the separation of the berberine. The syrupy residue is dissolved in about five times its weight of ethanol or isopropanol, and enough alcoholic hydrochloric acid is added to ensure a slight acid reaction to congo test paper after prolonged stirring, but an excess of acid is to be avoided to keep the loss of berberine to a minimum. The berberine hydrochloride, after filtration, can be washed with a small amount of alcohol and then with water. From the combined motherliquors and washings, the alcohol can be recovered by distillation, and the residue can be discarded.

Extraction of Berberine by Means of Water

After the powdered golden seal root has been exhaustively extracted with toluol, and all the toluol has been removed, the berberine can be



extracted. As mentioned before, wooden vessels are recommended for the extraction with water. When iron or other metal extractors are used, the berberine, on one hand, will be contaminated with metals, and the extractor, on the other hand, will be contaminated with berberine and other pigments which subsequently are hard to remove. Most suitable for wooden extractors are large, strong barrels or any other strong and tight wooden tanks. When barrels are used, they are stood on end, and the top is removed. A hole is then drilled as close to the bottom as possible, and a wooden stopcock is inserted tightly. Six to eight inches of loose excelsior is then placed evenly at the bottom and covered with a layer of burlap or other rough canvas, which should be turned up on the edge all along the side walls. The golden seal is then put on top of this improvised false bottom, carefully at first to keep the burlap or canvas from shifting.

The drug is now covered completely with hot, dilute acetic acid (1%) and is allowed to macerate overnight. It is advisable to circulate the liquor somewhat, by drawing off some at the bottom and returning it to the top of the extractor. The next morning the liquor is drained completely and should be filtered if it is not completely clear. The berberine hydrochloride can then be crystallized by the addition of a excess of hydrochloric acid, or the acid sulfate can be crystallized by the addition of an excess of sulfuric acid. The motherliquors from the hydrochloride can be discarded immediately; the motherliquors from the sulfate can first be treated with a small amount of hydrochloric acid to remove the last traces of berberine, after which they can be discarded. The crude berberine salts are filtered and are held for recrystallization.

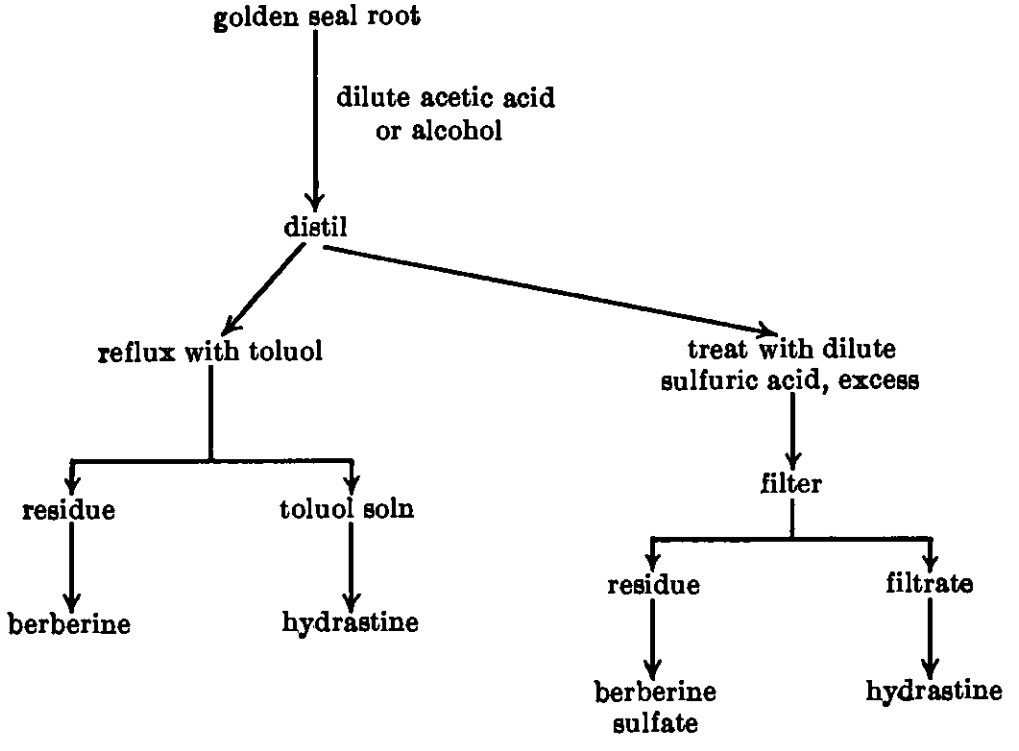
Extraction of the crude drug with hot, dilute acetic acid is repeated two to four times, until a sample of the liquid with hydrochloric acid shows only traces of berberine. The drug can then be discarded. Before recharging the extractor, it is advisable to discharge the excelsior and burlap and replace both with new material, especially the burlap, since the latter's pores may become clogged and slow down future extractions considerably.

Extraction of the Total Alkaloids from Golden Seal Root and Their Separation

The total alkaloids can be extracted from golden seal either by means of water or by means of alcohol, with the latter medium to be preferred. Extraction with alcohol can be undertaken on a countercurrent system, whereas extraction with water, which should be hot and slightly acidified (0.5–1% acetic acid), is best undertaken in the single batch system.

Both extracting media are evaporated under vacuo to a thick, syrupy residue. Care must be taken with the aqueous extract, since it may tend to foam at times when heated under vacuum.

The alkaloids can be separated and purified according to two different methods. The residue can be refluxed with fresh toluol several times to remove the hydrastine, and the berberine can be removed from the residue as described before; or, according to Wilhelm,[31] the syrup is treated with an excess of dilute sulfuric acid. Berberine acid sulfate is allowed to crystallize and is filtered off. The motherliquors are made alkaline with an excess of ammonia, and the mixture of hydrastine and canadine is either filtered off or taken up in ethylene dichloride, which is then dried and distilled under vacuum. Hydrastine can be obtained pure by recrystallizing the hydrastine-canadine mixture from ethyl acetate. The canadine will be found in the motherliquors.



Since the berberine acid sulfate obtained by Wilhelm's method (above) often contains hydrastine sulfate, the following modification has been introduced with much success: Enough hot, dilute (5%) sulfuric acid is added to the residue from the distillation to bring everything into solution. An equal volume of alcohol is then added, and the berberine acid sulfate is allowed to crystallize and is filtered off. From the motherliquors the hydrastine is separated as described before and is purified by crystallization from ethyl acetate or from a mixture of chloroform

and alcohol. The hydrochloride has been used instead of the sulfate with some success on the separation of the alkaloids.

Extraction of Berberine from Berberis

Berberine can be extracted from berberis (barberry root) either by means of hot water, hot, dilute acetic acid, or alcohol. In the first case, the hydrochloride or sulfate can be made directly from the clear extract, as described previously. In the second case the alcohol is evaporated, the residue is diluted with water, and the berberine is again crystallized from the clear liquor in the form of a salt, as described before. Wooden extractors are indicated for the aqueous extractions.

Extraction of Hydrastine from Golden Seal Leaves

Golden seal leaves contain 0.1–0.6% of hydrastine and a small amount of berberine. Although golden seal root is to be preferred as a source of these alkaloids, the leaves have been used with some success.

The dried leaves are powdered and are charged into the extractors, where they are treated with warm, dilute acetic acid (0.5–1.5%). Batches are extracted individually, three or four extractions are usually necessary, and the extractions are slightly warmed and circulated as usual, and are then allowed to macerate overnight.

The combined extractions are evaporated under vacuum to a thin syrup (care must be taken because of occasional foaming). The warm syrup is transferred to a mixing tank and is exhaustively extracted with toluol after having been made alkaline with ammonia. Because extraction with hot toluol is to be preferred, the occasional introduction of live steam can be recommended, although a jacketed mixing tank is to be preferred. Introduction of live steam has also been found helpful in breaking emulsions. The hydrastine is removed from the toluol with dilute sulfuric acid and can then be purified as described elsewhere.

METHODS OF PURIFICATION

Hydrastine base is fairly easily obtained pure and crystalline, but the salts are very hygroscopic and are difficult to obtain in a dry, crystalline state. Berberine is usually obtained in the form of its salts, which can be easily purified.

Hydrastine alkaloid, when obtained from its sulfuric acid solution by precipitation with ammonia, can be purified by recrystallization from warm acetone, methyl ethyl ketone,* or ethylacetate after treat-

* Private communication from Mr. R. L. Brown.

ment with charcoal and filtration. The base can be further purified by crystallization from a mixture of alcohol and chloroform.

Hydrastine hydrochloride can be obtained by two methods: The pure base can be dissolved in dry, alcoholic hydrochloric acid (methanol or ethanol), and the resulting solution can be evaporated to dryness in a vacuum desiccator in the cold, or the pure base can be dissolved in dry ether, free of alcohol, and dry hydrochloric acid gas can then be introduced until precipitation is complete. The hydrobromide and the sulfate are prepared similarly, but in preparing the latter an excess of sulfuric acid should be avoided.

Berberine is rarely obtained in the form of its base, the preparation of which is described elsewhere. The salts of berberine, including the acid sulfate, the hydrochloride, and the nitrate can be purified easily. The crude salts are dissolved in a sufficient amount of hot water, are treated with charcoal and filteraid or pulp, and the hot solution is then filtered through a stainless steel pressure filter. The filtered, hot, clear solution is then slightly acidified with the respective mineral acid, and the berberine salt is allowed to crystallize.

To aid in the filtration, filteraid or preferably pulp is generally used. Pulp, if it cannot be obtained as such, can easily be made by tearing several large sheets of soft filter paper into small shreds, and heating and soaking these in water until they begin to disintegrate. This pulp is then used after the water has been drained.

Any type of pressure filter can be used, including the one described in Chapter I, provided the filter and the pump is made of stainless steel or some other acid-resisting alloy. The filter must be thoroughly preheated immediately preceding its use, otherwise the berberine salts might crystallize prematurely. If necessary and possible, heating should be continued during the process of filtration. The hot, acidified filtrate should be stored in a glass-lined vessel, until it is sufficiently cool to be transferred to a stoneware crock without cracking the latter.

The berberine salts should be allowed to crystallize overnight in the cold and can then be filtered off and washed with a small amount of cold water and finally with acetone to afford quick drying. Washings and motherliquors can be discarded, although the washings could be held over for use in a succeeding recrystallization.

The neutral berberine sulfate is usually prepared from the berberine acid sulfate. Purified berberine acid sulfate is dissolved in the minimum amount of hot water and the solution is carefully neutralized by the addition of concentrated ammonia until the solution is neutral to litmus test paper. It is then allowed to cool and crystallize. A second crop of neutral berberine sulfate can be obtained by concentrating the motherliquors.

THE MANUFACTURE OF HYDRASTININE

Hydrastinine does not occur in the plant as such, but is produced either by pseudosynthesis from hydrastine or other alkaloids of lesser commercial importance, or it is completely synthesized.

Pseudosynthesis can be effected from hydrastine by oxidation with manganese dioxide [32] or with nitric acid. Only the oxidation with nitric acid, which is more feasible on a large scale, will be described:

One pound of pure hydrastine alkaloid is dissolved in ten times that amount of concentrated nitric acid (22%) and the solution is heated to 35–40°. At that point the oxidation will start and will proceed exothermically from then on. For that reason outside cooling and stirring are desirable. Cooling of the reaction mixture will indicate the end of the oxidation. The mixture is then cooled thoroughly and is extracted with ether to remove the opianic acid formed during the reaction. After three or four extractions with ether, the aqueous phase is neutralized with ammonia and the unchanged hydrastine is extracted with toluol or benzol. The yield of unchanged hydrastine usually varies from batch to batch. The neutral aqueous phase is then made strongly alkaline with a large excess of sodium hydroxide, but care should be taken to avoid a rise in temperature above 20°. The hydrastinine is then extracted with a large excess of ethyl ether (from which it may crystallize even during extraction) or benzol. The combined solvent extractions are distilled without further drying; the hydrastinine is allowed to crystallize from the residue (½–1 liters) overnight in the cold and is filtered off the next day. The crude base can be recrystallized from ether, benzol, or ethyl acetate, and it is then transformed into the chloride by treating with alcoholic hydrochloric acid and precipitating the chloride with acetone and ether. Hydrastinine chloride can be recrystallized from alcohol and ether.

A semi-synthesis of hydrastinine from berberine has been patented.[33] Berberine is reacted with a Grignard reagent to form alpha-alkyl-dihydroberberine, which can be reduced with tin and hydrochloric acid to the corresponding tetrahydroberberine. Methyl iodide followed by moist silver oxide yields des-N-methyl-alkyl-tetrahydroberberine, which, on oxidation with either potassium dichromate, nitric acid, or manganese dioxide, yields hydrastinine.

The most convenient method of preparing hydrastinine is its total synthesis from piperonal. This method has been thoroughly discussed in the literature, and, in Germany, numerous patents have been issued, protecting one or more of the intermediate steps. One of the key substances of this process is homopiperonylamine, which can be obtained

either from piperonal through the corresponding nitrostyrol,[34] or from safrole by oxidation with ozone or potassium permanganate [35] to homopiperonal and thence through homopiperonaloxime.[36] Hydrastinine can be obtained from homopiperonylamine either through the formyl-homopiperonylamine and nor-hydrastinine,[37] or through N-methyl-homopiperonylamine [38] and N-formyl-methyl-homopiperonylamine.[39] The latter can also be obtained directly from formyl-homopiperonylamine.[40]

The following method of preparing homopiperonylamine has been found to give excellent results:

Piperonal is dissolved in five times its weight of dry methanol, and the solution is cooled with ice. A 10% excess over the theoretical amount of nitromethane is added slowly (theory equals molecular proportions). One half hour after addition is complete, a 30% solution of potassium hydroxide in methanol is added in a 50% excess over theory (1½ times molecular proportions), and the mixture is poured in a thin stream under constant stirring into an excess of 15% hydrochloric acid. The nitrostyrol is filtered off, washed with water, and dried under vacuum.

The dry nitrostyrol is then suspended in ten times the amount of dry methanol, and a solution of one half that amount of sodium in methanol is then added. When all the material is in solution, glacial acetic acid is added (1.3 parts), the alcohol is removed under vacuum, and the methoxynitrostyrol is recrystallized from methanol.

The compound is dissolved in five times its weight of 90% formic acid, and to the solution is added under cooling twice the amount of zinc dust which had previously been treated with a solution of copper sulfate. When all the zinc has been added, cooling is discontinued, and the mixture is heated to 60° for 5 hr. The zinc is then filtered off, washed with formic acid and dilute methanol, and the combined filtrate and washings are evaporated under vacuum. From the residue, homopiperonylamine is liberated in the usual manner.

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

Hydrastine upon administration in larger than usual doses causes convulsions, which may be followed by partial or full paralysis, and death may occur from respiratory failure. These symptoms are due to its stimulation of the bulbar and of the spinal centers, as well as a somewhat depressant effect on the spinal cord and some action on the peripheral nerves. In the frog the symptoms of poisoning by hydrastine are like those of strychnine. According to at least one report, the intra-

venous injection of hydrastine causes a fall in blood pressure together with a depressant action on the muscles of the heart,[41] while according to another source the blood pressure is raised because of its central action. Raymond-Hamet and Mercier report no depressant action by subcutaneous injection.[42] Hydrastine is reported to increase intestinal contractions and to stimulate the muscle of the uterus.[43] As an internal styptic in uterine hemorrhage, hydrastinine is to be preferred because of its weaker action on the heart and the central nervous system.

Hydrastine as well as hydrastinine are excreted unchanged with the urine. The latter, like the former in large doses, also paralyzes the nervous system. A solution of hydrastinine causes dilatation of the pupil of the eye. The main use of hydrastinine today is that of an oxytocic styptic.

The physiological action of berberine is much weaker than that of hydrastine, but in large doses it too may be fatal. With increasing dosage it causes some stimulation of the heart,[44] some secondary depression, increase of the heart rate, and a fall in the blood pressure. Larger doses depress the heart.[45] Because of its toxicity to lower forms of life,[46] berberine is used in the treatment of the oriental sore. Because of its intense bitter taste, it is used in bitter tonics and in stomachics. Berberine is also used in eyewashes.

ANALYTICAL METHODS

Hydrastine in concentrated sulfuric acid yields no color in the cold, but turns dark red or purple on warming, whereas berberine under similar circumstances yields a greenish-yellow coloration. Addition of oxidizing agents, such as manganese dioxide, gives with hydrastine a yellow color which turns red and finally yellow again. Its solution in concentrated nitric acid is orange. Berberine under similar conditions yields a brown-red color. Concentrated sulfuric acid with potassium arsenate yields a yellow color with hydrastine; with berberine a similar yellow color turns greenish on standing. Hydrogen peroxide and sulfuric acid give a brownish-red with hydrastine but a dark cherry-red color with berberine.

Hydrastine in dilute hydrochloric acid is precipitated with potassium ferricyanide, mercuric chloride, and auri- and platinichloride. Amorphous precipitates are obtained, even from very dilute solutions, with picric acid, trinitrothymol, and hexanitrodiphenylamine. Paranitrophenyl propiolic acid yields microcrystalline needles.

According to Rosenthaler,[47] the following reactions are characteristic of hydrastine hydrochloride:

When placed in a solution of Erdmann's reagent, large amounts of much-branched needles are obtained which are partly curved. With hexanitrocobalti-sodium, spherical crystals with small rosettes or long aggregates of fine slender threads are obtained. Potassium iodide yields rods, spears, and plates. Mercuric chloride produces needles and spears, often with a saw-like margin, less often of rhombic shape. Sodium perchlorate gives spears and plates, often with a curved margin.

Hydrastinine is not precipitated from its aqueous solution with sodium bicarbonate, sodium carbonate, or ammonia, but the addition of sodium or potassium hydroxide causes the base to crystallize. Mercuric chloride gives fine needles. Potassium ferrous cyanide yields yellow-greenish leaflets or brown prisms. Potassium dichromate gives yellow plates, and potassium permanganate yields a purplish crystalline precipitate.

Berberine yields crystalline nitrates and sulfates, chloroaurates, and chloroplatinates. The iodide and the periodide are also characteristic, as is its crystallizate with acetone. Most of its color reactions have been mentioned previously. Potassium dichromate yields an amorphous yellow precipitate; with meta-dinitrobenzoic acid needles are obtained.

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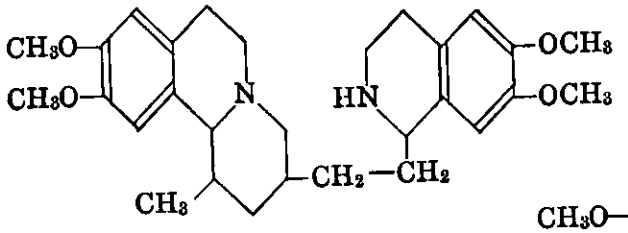
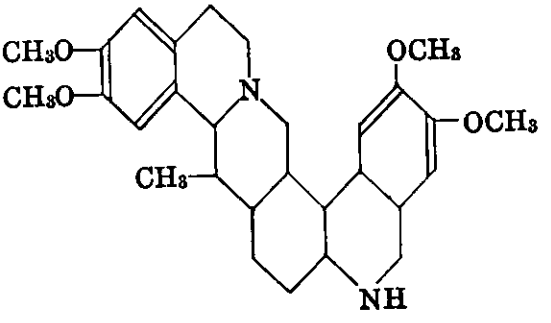
CHAPTER XI

THE ALKALOIDS OF IPECACUANHA

CHEMISTRY

The important alkaloids found in *Cephaelis ipecacuanha* are emetine and cephaeline. The structure of emetine has not been ascertained as yet, but the formulae presented here have been suggested.



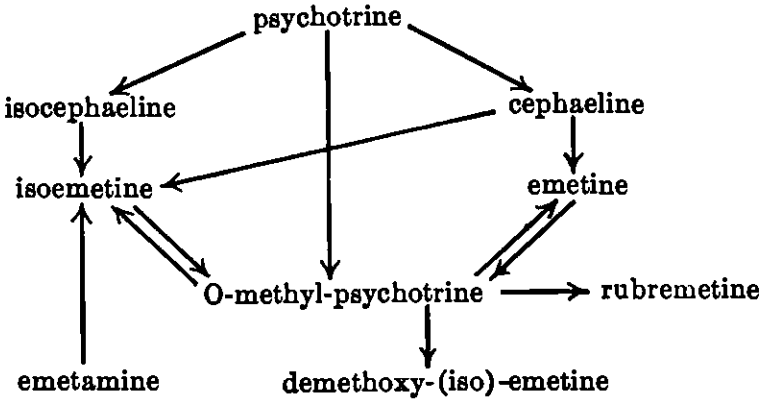


Other alkaloids are also present, notably psychotrine, O-methyl-psychotrine and emetamine. All these alkaloids are closely related to each other and to emetine and cephaeline as indicated below.

psychotrine → cephaeline
↓ ↓
O-methyl-psychotrine → emetine

O-Methyl-psychotrine can be transformed into psychotrine by partial demethylation with hydrochloric acid at 170°.[1] On the other hand, psychotrine can be methylated with methyl sulfate to O-methyl-psychotrine.[2] Other transformations can be brought about involving either methylations of hydroxyl groups or hydrogenations, and a number of new isomeric bases have been synthesized in this manner. None of these new bases occur in nature. Psychotrine can be reduced to a mixture of two stereoisomerides, cephaeline and isocephaeline (m.p.:

160°),[3] which can be methylated with methyl sulfate to emetine and isoemetine (m.p.: 98°) respectively.[4] Reduction of O-methyl-psychotrine gives rise to emetine, isoemetine,[5] and demethoxyemetine (or demethoxyisoemetine). Emetamine seems to be related to emetine, but it contains two double bonds which can be reduced.[2] The table showing the relationships of all these bases can then be detailed as follows:



None of the five alkaloids occurring in nature contain a methylimino group. Emetamine is the only base containing two tertiary nitrogen atoms; all others contain one tertiary and one secondary nitrogen atom.[6] Both emetine and cephaeline can be demethylated to the same phenolic base, $C_{25}H_{32}O_4N_2$, which has been given various names such as emetoline.[7]

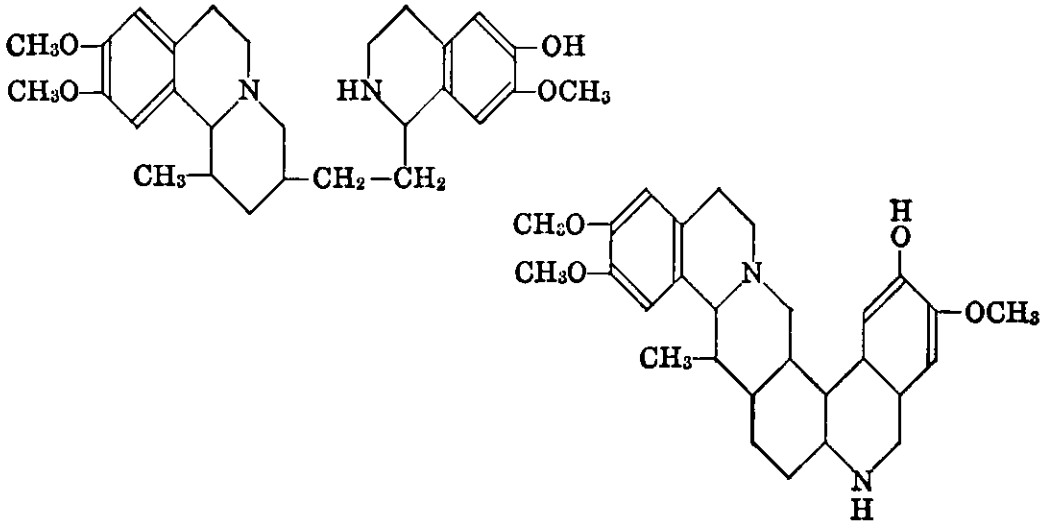
A by-product obtained by the careful methylation of cephaeline to emetine with methyl sulfate or sodium methyl sulfate, are N-methyl-cephaeline (m.p.: 195°) and N-methyl-emetine, which has also been obtained from emetine directly. Exhaustive methylation of emetine with methyl iodide gives rise to two stereoisomeric N-methyl-emetine methiodides (m.p.: 226° and 262°), which can be reduced to the same N-methyl-emetine-methine, $C_{32}H_{46}O_4N_2$. N-methyl-isoemetine-methine has been obtained by a series of similar reactions.[8]

Gentle oxidation of emetine and isoemetine, $C_{29}H_{40}O_4N_2$, yields O-methyl-psychotrine, $C_{29}H_{38}O_4N_2$, but more vigorous oxidation gives rise to a new compound, rubremetine, $C_{29}H_{32}O_4N_2$, which contains one quaternary nitrogen atom and has been isolated as rubremetinium chloride.[9] The second nitrogen atom in that compound is no longer basic. Still stronger oxidation yields 6,7-dimethoxy-isoquinoline-1-carboxylic acid and meta-hemipinic acid.[10] Oxidation under somewhat different conditions gave rise to 1-keto-6,7-dimethoxy-1,2,3,4-tetrahydro-isoquinoline,[11] suggesting the possibility of the presence of two isoquinoline nuclei in emetine. This tetrahydro-isoquinoline part of emetine

was shown to contain the secondary nitrogen atom, because oxidation of N-benzoyl-emetine gave rise to 3,4-dimethoxy-6-beta-benzamido-ethylbenzoic acid.

That emetine is the methyl-ether of cephaeline has been shown previously. Evidence of the location of the free hydroxyl group in cephaeline was provided by an examination of the products of oxidation of cephaeline ethyl ether, which yielded a mixture of 1-keto-6,7-dimethoxy- and 1-keto-6-ethoxy-7-methoxy-1,2,3,4-tetrahydro-isoquinoline.[11]

Formulae for emetine and the other alkaloids have been suggested by Robinson,[12] by Brindley and Pyman,[13] and by Staub.[14] These formulae were developed by taking into account the reactions mentioned previously, and the possible phytochemical origin of the alkaloids. Most of the chemical transformations can be satisfactorily explained by one of the formulae of emetine given previously and by one of the formulae of cephaeline given below:



For details of the reasoning leading to the formation of these formulae, the original literature should be consulted.

Emetine, $C_{29}H_{40}O_4N_2$, has been obtained as a white amorphous powder,[3] soluble in alcohol, ether, or chloroform, less soluble in benzol or petroleum ether, and sparingly soluble in water. The optical rotation in chloroform is independent of concentration, but it varies with the latter in 50% alcohol from α_D −25.8–32.7; (α_D −50 in chloroform). The hydrochloride has been crystallized from water,[15] but in that solvent it tends to form supersaturated solutions from which it separates amorphously. The hydrobromide is the salt usually employed in its purification. The hydriodide and the nitrate can be crystallized from either water or alcohol, but the sulfate is very soluble in water. The platinichloride is amorphous.

Cephaeline, $C_{28}H_{38}O_4N_2$ (m.p.: 120–130°), can be purified either through one of its salts such as the hydrochloride or hydrobromide, or by crystallization of the base from ether. The base is easily soluble in acetone, alcohol, and chloroform, and in aqueous solutions of alkalies, but sparingly in ether and petroleum ether. The hydrochloride and hydrobromide are more pronounced crystalline than the corresponding salts of emetine, but the sulfate, hydriodide, and nitrate are amorphous.

Psychotrine, $C_{28}H_{36}O_4N_2$, crystallized from dilute acetone or alcohol with four moles of water of crystallization. The base is soluble in acetone, chloroform, and alcohol, sparingly in ether and benzol. Most of its salts have been crystallized from aqueous solutions.

O-Methyl-psychotrine, $C_{29}H_{38}O_4N_2$, has been isolated by Pyman [2] from the motherliquors of emetine in the form of its acid oxalate, in which form it crystallizes together with emetamine, from which it was separated by treating the combined bases in chloroform with dilute sulfuric acid. Its salts are crystalline, and their aqueous solutions are fluorescent. The base has also been described by Brindley and Pyman.[18]

Emetamine, $C_{29}H_{36}O_4N_2$, has been crystallized from ethyl acetate and from ether, from the latter with one half mole of solvent of crystallization. The base is soluble in alcohol, benzol, and chloroform, sparingly in ether, and insoluble in alkalies.

		Emetine	Cephaeline	Psychotrine	O-Methyl Psychotrine	Emetamine
Base	m.p.	74	116, 130	120–138	124	154
	α_D	−25.8– −50	−43.4		+43.2	+13.6
Hydrochloride	m.p.	235–255	245–270			80, 223
	H_2O *	7	7			8.5
	α_D	+11, +21, +53	+25–+29.5			−17.5
Hydrobromide	m.p.	250–260	266–293		200	225
	H_2O *	4	7		4	7
	α_D	+12–+15			+48	−22– −24.3
Hydriodide	m.p.	235–238		200–222		208, 274
Nitrate	m.p.	188–245		187		166
	H_2O *	3		1		2
Sulfate	m.p.	205–245		217	247	
	H_2O *	7		3	7	
Platinichloride		253–265				
Acid oxalate				130-145	150-162	172

* Water of crystallization.

NATURAL SOURCES

According to the *U. S. Pharmacopœia* (8th Edition), "Ipecac consists of the dried rhizome and roots of *Cephaelis ipecacuanha* (Brotero)

A. Richard, known in commerce as Rio or Brazilian Ipecac, or of *Cephaelis acuminata* Karsten, known in commerce as Cartagena, Nicaragua, or Panama Ipecac (Fam. Rubiaceae). Ipecac yields not less than 2% of the ether soluble alkaloids of Ipecac. It contains not more than 5% of its overground stems, and not more than 2% of other foreign matter." Only the Rio Ipecac is recognized in Great Britain, where a somewhat different limit is also set on the different organic impurities and on the content of total alkaloids and the ratio of emetine to cephaeline.

The alkaloids are also found in other plants besides Ipecac root,[14,16] in Indian Ipecac root,[17] and in Ipecac seeds.[18]

Brazilian Ipecac root is by far the most desirable source for the manufacture of emetine, because the main portion of its total alkaloid content consists of emetine, whereas in the Nicaraguan Ipecac root cephaeline is the main alkaloid, necessitating its further conversion into emetine by O-methylation.

The Rio or Brazilian Ipecac grows in moist, dense forests in Brazil and Bolivia, but is also found in Colombia. The plant is a low, straggling shrub. Parts of the stem are often underground, and are then collected together with the slender, smooth rhizome, which grows to different kinds of roots. Some roots are wiry and annulated, others are smooth and slender. The rhizome is usually horizontal. The stem of the plant is green, angular, and somewhat short. It grows few opposite, obovate, entire leaves. The flowers are small and white. The fruits are purple berries, which grow in clusters and contain two seeds each.

Nicaraguan Ipecac is found in the damp and dark forests of Colombia. It closely resembles the Rio Ipecac, and it has been claimed at various times that the differences between the two species are merely geographic and not botanical, as stated by the *U. S. Pharmacopœia*. Nicaraguan Ipecac is said to be somewhat thicker, and to have less pronounced annulations. Nicaraguan Ipecac occurs in commerce in two varieties, the grayish and the reddish variety. The latter root shows transverse ridges and numerous abrasions of somewhat lighter color.

The roots and rhizomes are collected between January and the end of March. They are washed free of soil, dried in the sun, and are shipped in bags. It has recently been stated, in reference to Indian Ipecac root, that three-to-four-year-old plants give the highest yield of emetine.[19] The alkaloids are said to be situated in the peripheral cells just under the cork.[20]

Detailed descriptions of both varieties of Ipecac root will be found in the *U. S. Pharmacopœia*. They can be distinguished by (a) their out-

ward appearance, Rio Ipecac is more reddish-brown, while the other is of grayish-brown and shows fewer annulations; (b) their histological appearance, by the difference in the size of the starch grains and in the cells of the woody portions; [21] and (c) their content of alkaloids, or, more specifically, the ratio of emetine to cephaeline.

Ipecac root is often subject to adulterations and substitutions, the most common of which are the following:

a. Ipecac Stems. They can be recognized by the presence of leaf scars, and, microscopically, by the presence of many chloroplasts. The stem, and especially its underground part, contains considerably less alkaloid than the roots and rhizome.[22]

b. Undulated Ipecac. This root is derived from *Richardsonia scabra* St. Hilaire, found in Brazil. The root is less annulated, shows fewer fissures, and the bases of several small stems. The bark is often purplish, the wood porous.

c. Less Striated Ipecac. It is now thought to be derived from *Manettia ignita* Schum.[23] Short, cylindrical fragments, of grayish-brown color. Irregular constrictions, starchy cross section of purplish color. It is said to contain emetine.

d. Greater Striated Ipecac. The root is derived from *Psychotria emetica* Linné, resembles Nicaraguan Ipecac but can be distinguished by the absence of starch.

e. White Ipecac. Obtained from *Ionidium ipecacuanha* St. Hilaire, found in Brazil. The many-branched root shows no annulations, is grayish-white or yellowish, is porous and woody, distinguished also by the absence of starch.

f. Trinidad Ipecac. This root, which is almost white internally, is void of the alkaloids of Ipecac. It is derived from *Asclepias curassavica* Linné.

A voluminous literature concerning the adulteration of Ipecac and their detection has been published by Hartwich,[24] Greenish, [25] Schneider,[26] and Kraemer.[27]

Average contents of alkaloids will be found in the table below.

		Percentage of Total		
Root	*Total Alkaloids (%)*	*Emetine*	*Cephaeline*	*Psychotrine*
Brazil.....	2.5 *	72	26	2
Nicaraguan....	2	40	57	3

* Average analysis: 1.35% emetine; 0.26% cephaeline; 0.04% psychotrine; 0.002–0.006% emetamine; 0.015–0.033% O-methyl-psychotrine.

MANUFACTURING METHODS

It is important to mention certain precautions which should be observed in the manufacture of emetine. It has been found that emetine, cephaeline, and to a lesser extent powdered Ipecac can cause very painful inflammation of the eyes, the nose, and the skin under the fingernails. Absolute cleanliness, efficient ventilation, and protection of eyes and nose, especially when handling dry emetine hydrochloride, are an absolute necessity. Asthmatics are said to be especially sensitive toward Ipecac.*

Ipecac root is powdered and charged into the extractors as described in Chapter I. The drug is then extracted with either methanol, 50% methanol, or 70% denatured alcohol. These solvents have been found to yield good results. Circulation of the menstruum and heating, as well as countercurrent extraction, are indicated. Acidification of the alcohol or the dilute alcohol prior to extraction is not necessary. The total extracts are combined and evaporated, first under ordinary pressure and finally under vacuum. The residue remaining in the still is a dark brown, almost black, heavy and thick syrup. While still warm, it is mixed with an equal volume of warm water, stirred until all is in solution, and the contents of the still are then removed to an iron mixing tank, which should be placed either in the open air or in a well-ventilated building. It should be equipped with an efficient stirrer.

This aqueous solution is now ready for the isolation and separation of emetine and cephaeline. It is made strongly alkaline by the addition of a large excess of concentrated aqueous ammonia, and the alkaloids are removed with a water-immiscible solvent. Toluol cannot be recommended because of the relatively slight solubility of the alkaloids in this solvent even at somewhat elevated temperatures. The only solvents to be considered are isopropyl ether and water-immiscible ketones, such as methyl isobutyl ketone. Both solvents have been found to yield equally good results. Isopropyl ether might have a somewhat higher preferential solubility toward the alkaloids. Repeated extractions with the solvent are necessary to remove all the alkaloids from the crude extract. It has been found advantageous to undertake the extractions at somewhat higher temperatures. Heating can be accomplished either by means of direct live steam or through a jacket. Prolonged and efficient stirring at each extraction is necessary. Separation of the solvent occurs readily, and no formation of emulsions has been observed. It has been found easiest to syphon off the solvent from the top. The

* Private communication from Mr. R. L. Brown.

solvent is, of course, used over again after the alkaloids have been removed from it. When a sample of the solvent, on extraction with dilute hydrochloric acid, yields only a slight cloudiness with a large excess of Mayer's reagent, the extractions can be considered finished, and the ammoniacal aqueous phase can be discarded.

The solvent phase, after separation from the extract, is first treated twice with a small amount of dilute (10–15%) solution of potassium hydroxide, to separate the cephaeline from the emetine and also to remove other phenolic bases. The second alkaline extraction is kept aside and used as a first extraction on the following batch. These extractions are undertaken in the cold, in an iron extractor with bottom outlet, or in a stoneware separator. The alkaline extractions are combined and collected. The cephaeline is later isolated and transformed into emetine.

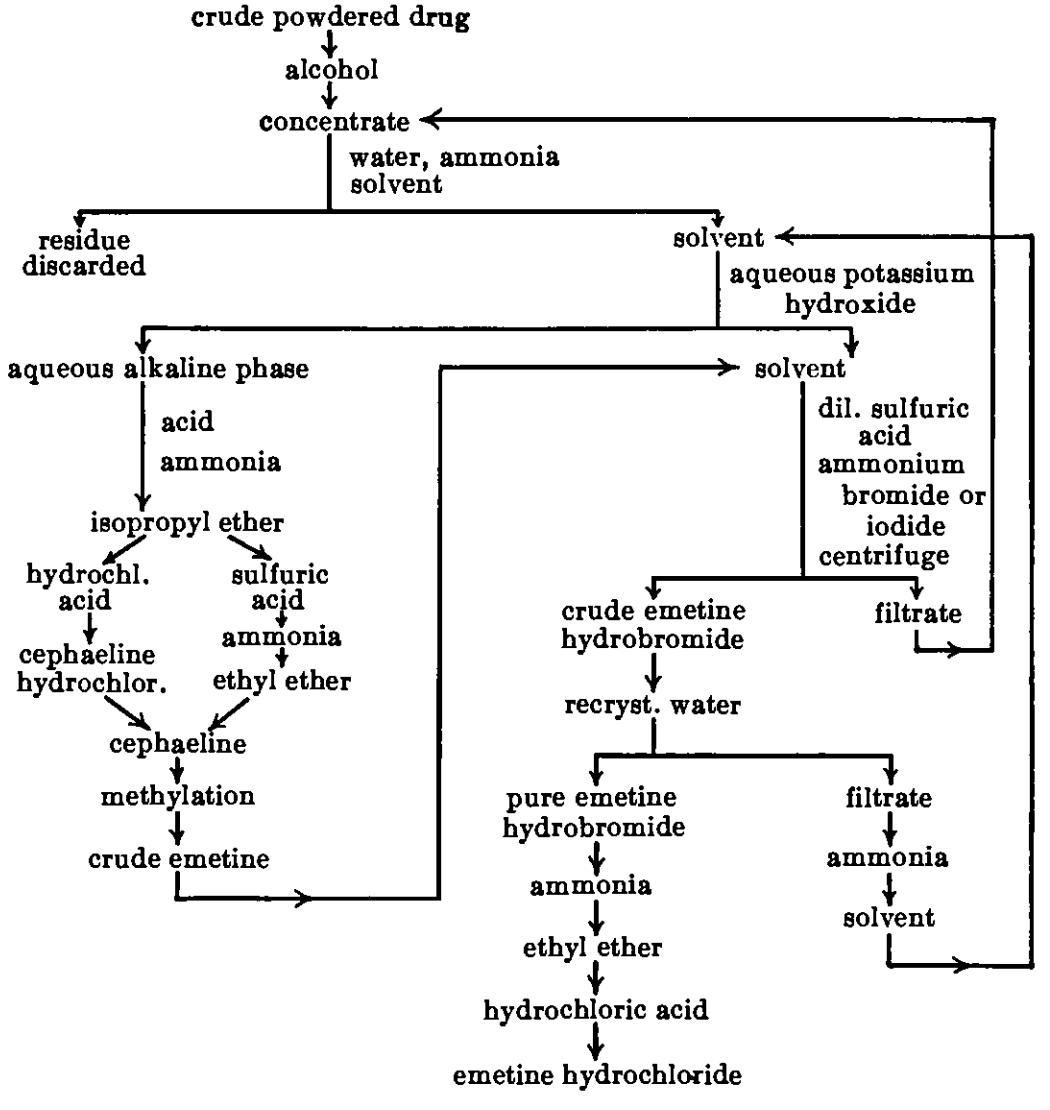
The solvent solution, which is now free of phenolic alkaloids, is transferred to a glass-lined or stoneware separator, where it is first washed with a small amount of water. This washwater, after it has been separated, is used for the preparation of the dilute solution of potassium hydroxide. The solvent is then extracted with a small amount of dilute sulfuric acid (15–20%). Too large an excess of acid should be avoided. One extraction is usually sufficient to remove all the emetine from the solvent, provided the stirring has been effective enough. The solvent, which is now void of alkaloids, is returned to the warm, ammoniacal extract for further removal of alkaloids.

The acid extractions, containing the emetine, are combined and the alkaloid is crystallized and purified either through the hydrobromide or through the iodide. A concentrated solution of either ammonium bromide, or another salt of hydrobromic acid, or of ammonium iodide or another salt of iodic acid, is added in an excess under constant stirring. To keep the solution concentrated, the solid salts can also be added under stirring. The original sulfuric acid solution of emetine should not be too strongly acid; if necessary it should be neutralized by the careful addition of ammonia until it is just acid to congo test paper. The emetine hydrobromide or hydriodide is allowed to crystallize for at least 24 hr, preferably longer in the case of the hydrobromide.

When all the emetine has crystallized in the form of one of these fairly insoluble salts, it is filtered or centrifuged off. Centrifuging is especially recommended for emetine hydrobromide because of the slimy nature of that compound. The precipitates are washed with small amounts of ice cold water until they are almost free of color. The filtrate and washings are combined, made alkaline with ammonia, extracted with a

solvent, and the solvent is used over again in the process as indicated in the diagram.

This crude emetine hydrobromide is now dissolved in boiling water, a little at a time, until the boiling solution is almost saturated. Boiling is then discontinued, and charcoal and filteraid are added to the somewhat cooled solution to avoid possible boiling over. The solution is then brought to boiling again, and it is then filtered through the pressure filter described in Chapter I. The filtercake is washed with a small amount of boiling water. It is not discarded, however, because the charcoal adsorbs considerable amounts of emetine hydrobromide which are only difficultly removed therefrom. The clear filtrates are allowed to crystallize for 24 hr, and the emetine hydrobromide is then centrifuged off and washed with a small amount of cold water. The combined filtrate and washings are made alkaline with ammonia, extracted with a solvent which is used further as shown in the diagram.



The crude emetine hydriodide can also be recrystallized from a large amount of boiling water or from hot alcohol. If the latter is used, the filtrate and motherliquors are combined, distilled under vacuum to a small volume, diluted with water, and returned in the process to the crude extract.

This recrystallized emetine hydrobromide or hydriodide is now sufficiently pure to permit its transformation into emetine hydrochloride.

The recrystallized emetine hydrobromide or hydriodide is suspended in a small amount of warm water, mixed with a large amount of ethyl ether, and the base is liberated by the addition of an excess of ammonia. The use of warm water is recommended to facilitate liberation of the free base and its solution by the solvent. The aqueous ammoniacal phase is extracted once more with ether, and it can then be reused in a concentrated extract. The ether solutions are combined, washed once with a dilute solution of sodium hydroxide (1–2%), and then twice with water, which is carefully separated each time. Any alkaloid which has not dissolved in the ether can be removed from the vessel, dissolved in a small amount of warm alcohol, and can then be dissolved in the ether without difficulty.

The washed, ethereal solution of emetine alkaloid is then extracted with hydrochloric acid which has been diluted with distilled water two to one. The neutralization must be undertaken with efficient stirring, and the acid must be added slowly, because an excess must be avoided. The final solution should be just slightly acid to congo test paper. Emetine hydrochloride often will begin to crystallize even before all the acid has been added. To avoid this it has been found helpful to add a small amount of distilled water prior to adding the acid. When all the emetine has thus been converted into its hydrochloride, the ether is removed, either by syphoning it off, or by separating the aqueous solution of the emetine hydrochloride in a large glass or glass-lined separator, the valve of which should also be glass-lined or of stainless steel. The emetine hydrochloride is permitted to crystallize in a dark, cold spot for at least 24 hr. It is then filtered off, but the crystalline precipitate is only drained well on the filter and not washed. It is finally permitted to air-dry in a dark place at room temperature. The motherliquors are concentrated under vacuum, and further crops of emetine hydrochloride are obtained in a similar manner.

Emetine hydrochloride has also been manufactured in a slightly different manner. The ethereal solution of emetine was extracted rapidly, and with efficient stirring, with concentrated acid which had been diluted with distilled water in the ratio 1:1. The emetine salt was

permitted to crystallize from this solution after most of the ether had been removed, and crystallization was usually complete within 1–2 hr. This semisolid crystalline mass was then cut up by hand and spread on glass trays, where it was permitted to air-dry until the moisture content permitted by the *U. S. Pharmacopœia* was reached. Drying of the material was facilitated by further reduction of the size of the pieces of emetine hydrochloride from time to time.

The dry emetine salt is then powdered by means of a mill, but extreme care should be taken to protect the eyes, nose, and fingernails.

To make the manufacture of emetine economically successful, it is imperative to convert the cephaeline into emetine. For that purpose, the cephaeline, which has been obtained in the form of a solution in potassium hydroxide, must first be purified. The alkaline solution is neutralized or made slightly acid with sulfuric or hydrochloric acid, under cooling, and the cephaeline is then extracted after making alkaline with ammonia. The solvent used in this operation can be either ethylene dichloride, isopropyl ether, or methyl isobutyl ketone. When all the cephaeline has been removed from the aqueous phase, it can be discarded. The alkaloid is then re-extracted with dilute sulfuric or hydrochloric acid. To introduce an additional step of purification, it has been found advantageous to perform this extraction with an excess of hydrochloric acid, filtering this solution warm with charcoal, and allowing the hydrochloride to crystallize for several days. After filtering, the filtrate can be made alkaline with ammonia, the cephaeline re-extracted with isopropyl ether or another solvent, and can then be crystallized again as the hydrochloride. The salt of cephaeline can be used as such, or it can be transformed into the base in the usual manner. If the above-mentioned solvent solution of cephaeline appears to be relatively free of impurities, the additional purification through the hydrochloride can be avoided. The cephaeline is then extracted with dilute sulfuric acid, which is treated with charcoal and filteraid, made alkaline with ammonia, and extracted with ether. Cephaeline base is obtained after drying the ethereal solution with anhydrous sodium sulfate, followed by evaporation to dryness at a low temperature.

The O-methylation of cephaeline can be accomplished by a number of different methods, some of which have been the subject of patents.[28] The following methylating agents have been used successfully: Dimethyl sulfate, sodium methyl sulfate, trimethylphenyl ammonium hydroxide, nitrosomethylurea and nitrosomethylurethane (ethyl-N-nitroso-N-methyl-carbamate).

a. Methylation with Dimethyl Sulfate. 18.5 parts of sodium are dissolved in about 400 parts of dry isopropanol, and to the solution is added 100 parts of cephaeline hydrochloride. After the sodium chloride has been filtered off, 20 parts of dimethyl sulfate are added. The solution is warmed to about 60° in a closed vessel for several hours. The isopropanol is then removed under vacuum, and the residue, which must not be subjected to extensive heating to avoid hydrolysis, is processed similarly to a similar concentrated extract of Ipecac root.

b. Methylation with Sodium Methyl Sulfate. This methylating agent can be prepared in the usual manner. 100 parts of dry methanol are refluxed with 71 parts of concentrated sulfuric acid for 3–4 hr. The mixture is then diluted with twice its volume of water, an excess of powdered calcium carbonate is added, the mixture is filtered, the clear filtrate is treated with sodium carbonate until slightly alkaline, filtered, concentrated under vacuum, and allowed to crystallize. All precipitates are washed with a small amount of methanol. The yield of sodium methyl sulfate is approximately 50–60 parts. To effect the methylation of cephaeline, 1 lb of sodium is dissolved in ten times its weight of dry amyl alcohol (fusel oil) under refluxing. To this solution is then added 10 lb of cephaeline and 4 lb of dry sodium methyl sulfate, and the mixture is refluxed for about 6 hr. The solvent is then distilled, and the residue is treated as described before. Instead of distilling, the emetine and unchanged cephaeline can be extracted with dilute hydrochloric acid. This method gives a large proportion of N. methyl cephaeline.*

c. Methylation with Trimethylphenyl Ammonium Hydroxide. 13 lb of dimethylaniline is mixed with 20.4 lb (hydrated) or preferably 18.5 lb of dry *p*-methyl-toluenesulfonic acid and this mixture is added to a solution of 2.48 lb of sodium in dry methanol or anhydrous ethanol or isopropanol. The sodium sulfonate is filtered off, and to the clear filtrate is added 50 lb of purified cephaeline. After warming for some time, the alcohol is evaporated and the residue is treated further to isolate the emetine and unchanged cephaeline. This method has given excellent yields (96% of theory).

d. Methylation with Nitrosomethyl Urea or Nitrosomethyl Urethane. The preparation and use of these reagents has been described extensively in the literature, and also in *Organic Syntheses*, Vol. II (1943), pp. 461, 464. The urea derivative cannot be recommended for use on a large scale, mainly because of its extreme sensitiveness to temperatures above 20°. The urethane derivative is to be preferred. It can be prepared

* Private communication from Mr. R. L. Brown.

fairly easily, and it can be stored in the icebox until ready for use. The method of its application is also well known, but the following example will serve as an illustration. The cephaeline hydrochloride (1 kg), or its equivalent weight as base, is dissolved in about 10 liters of dry methanol, the solution is cooled, and about 1 liter of nitrosomethyl urethane is added, followed by about 6.5 liters of a 5% solution of sodium hydroxide in dry methanol, which is added slowly and with efficient cooling and stirring. The sequence of addition of the alkali and the methylating agent can be reversed. After several hours, half that amount of nitrosomethyl urethane is added, followed by half the amount of sodium hydroxide solution. After several hours of stirring and cooling, the alcohol is removed under vacuum, and the residue is worked up as described before. This method is being used technically on a very large scale.

Nitrosomethyl urethane was found to have toxic properties. Contact with the skin and inhalation of its vapors must be avoided.

In all of the above-mentioned procedures, any unchanged cephaeline will be found in the residues in the form of its sodium salt. The cephaeline can be liberated by acidifying first, then making alkaline with ammonia, extracting the emetine and cephaeline together, and separating them as described under the manufacture of emetine from Ipecac extract.

An average of 1.5% of emetine can be produced from Ipecac directly, while a further 0.5% can be obtained by conversion of cephaeline.

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

Emetine, when administered in small doses, acts as an expectorant and as an emetic, and it increases various secretions of the body. Together with opium it is used as a diaphoretic, and it is also said to have hemostatic powers. It is highly irritating to the gastric mucosa. Large doses injected subcutaneously or intravenously act as a depressant on the motor and respiratory center. Certain changes seem also to take effect in the lungs. It has been used in hemoptysis, hemophilia, and asthmatic emphysema.[29]

The chief use of emetine is that as a remedy in amoebic dysentery, when the hydrochloride is injected or the bismuth iodide administered orally. Emetine is without effect in the type of dysentery caused by a bacillus, but it is highly effective against *Entamoeba histolytica*, especially when the latter is embedded in the intestines and not free-swimming.[30] The drug may either act in the alimentary canal directly, or it may render the blood amoebicidal.[31] Emetine has also been used for the cure of hepatic abscesses [32] and of bilharziasis.[33]

Cephaeline acts similar to emetine, but it is more toxic and irritant.[34] Psychotrine is said to be inactive and nontoxic.[35] Synthetic emetine-substitutes have been investigated.[36]

Because of the slow elimination of emetine from the system, its toxic power accumulates. Some of the symptoms observed are: acidosis,[37] hemorrhages of different internal organs,[38] bloody diarrhea, and others.

ANALYTICAL METHODS

Emetine alkaloid gives a greenish-yellowish color with concentrated sulfuric acid and molybdic acid, but its hydrochloride produces a green color under similar conditions. Vanadium oxide and sulfuric acid also give rise to a green coloration. Hydrochloric acid gives no color; concentrated nitric acid gives an orange color.

Cephaeline with concentrated sulfuric and molybdic acid gives a dull greenish-blue color if a trace of hydrochloric acid is present. It also gives a characteristic reaction with *p*-nitro-diazobenzene.[39] Froehde's reagent and hydrochloric acid give a bright blue color.

Both alkaloids give precipitates with auric chloride, platinic chloride, phosphotungstic acid, Mayer's reagent, potassium bismuth iodide, and potassium iodide.[40] When bleaching powder is added to a solution of the alkaloids in dilute hydrochloric acid, orange colorations are produced.[41]

Psychotrine gives a green color with sulfuric and molybdic acid, a green color with Froehde's reagent and hydrochloric acid, and it also reacts with *p*-nitro-diazo-benzene.[39,41] Ferric chloride gives a dark precipitate or a blue coloration.

The microchemical identification of Ipecac root has been described by Rosenthaler.[42] Regarding quantitative determination of the alkaloids, the original literature should be consulted.[43]

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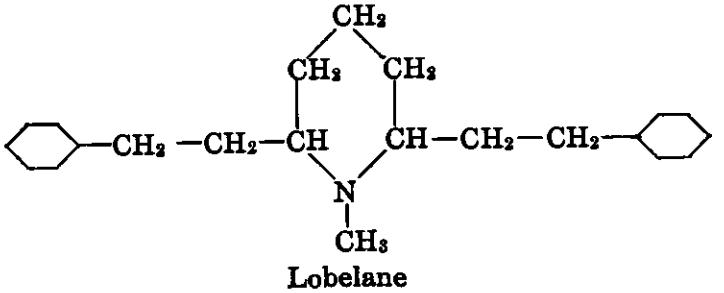
CHAPTER XII

THE ALKALOIDS OF LOBELIA

CHEMISTRY

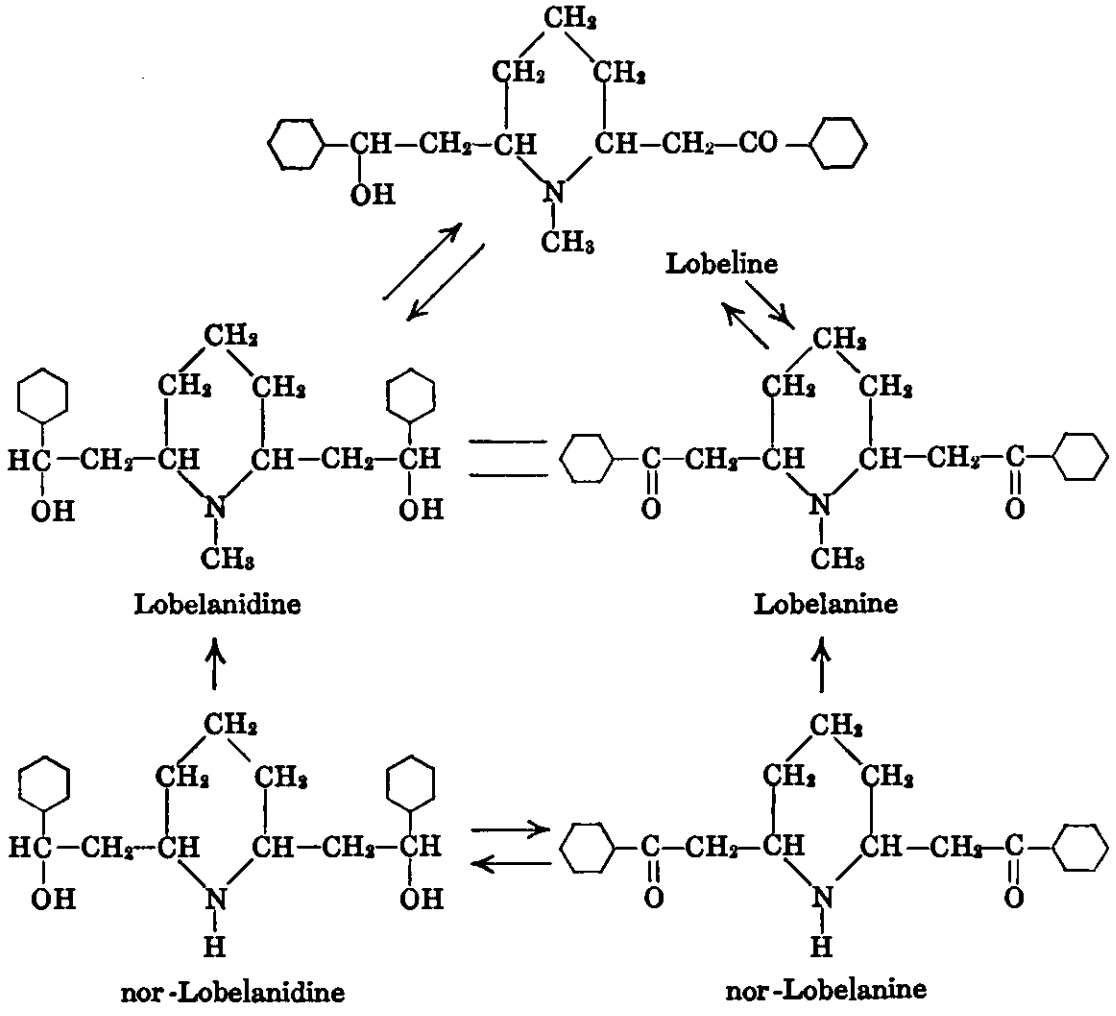
Because of recent discoveries in the field of medicine, the demand for lobelia alkaloids, and especially for alpha-lobeline, has risen considerably—so much so that the supply of lobeline from the natural source has been inadequate. Methods have been devised, however, to synthesize lobeline on a commercial scale. This chapter, therefore, shall be devoted not only to the methods of manufacturing the lobelia alkaloids from the natural drug, but also to methods of synthesizing them.

The principal alkaloid of *Lobelia inflata*, and the one which is physiologically most active, is alpha-lobeline, which is a derivative of piperidine, and to which the other alkaloids of lobelia can easily be correlated. Lobelanine is the corresponding diketo-compound, while lobelanidine is the corresponding dihydroxy-derivative. Interconversion can readily be effected by mild oxidation and reduction, respectively. Nor-lobelanine and nor-lobelanidine can be transformed into lobelanine and lobelanidine by N-methylation. The parent hydrocarbon, lobelane, from which the alkaloids are thought to be derived, does not occur in the natural drug but has been synthesized.[1]



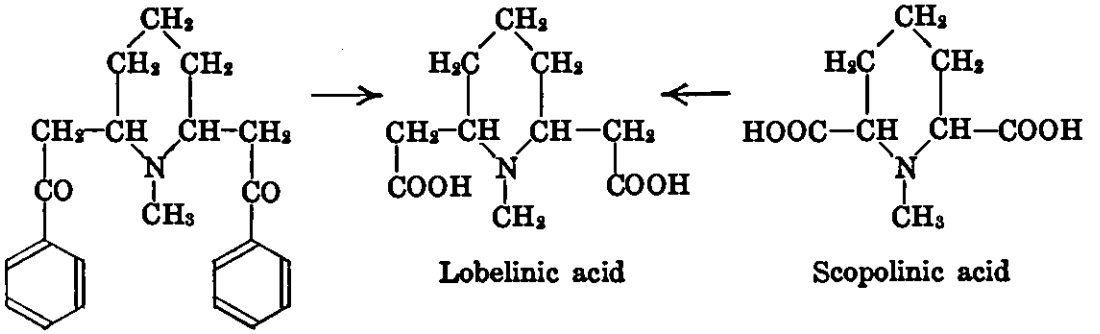
Lobelane

The presence of alkaloids in lobelia was first recognized by Procter [2] and by Lewis,[3] but it was not until fairly recently that the chemical constitution of the alkaloids was established and confirmed by synthesis.

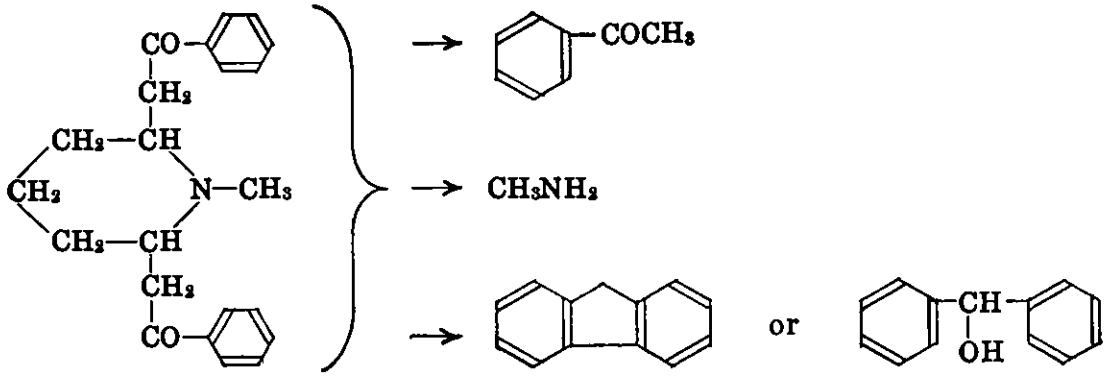


It was found that the five main alkaloids yield acetophenone on heating either with or without a catalyst, and because of their ease of interconversion their only difference was thought to be due to a hydroxyl or keto group. In the first formulae suggested, ether linkages were devised.[4] Difficulties were encountered when it was found that the quaternary base obtained from lobelanidine proved to be extremely stable against further degradation according to Hofmann. The same was true of the quaternary base obtained from the hydrocarbon lobelane.

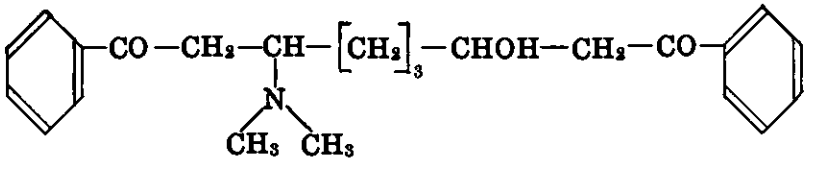
New evidence, however, was discovered in 1929 which finally helped to solve the problem. It was found that lobelanine hydrochloride upon treating with zinc dust gave more than one mole-equivalent of acetophenone, thereby excluding the presence of an ether linkage. The dioxime of lobelanine was prepared and subjected to the Beckmann rearrangement, yielding the dianilide of lobelinic acid, which was identified as 1-methyl-piperidine-2,6-diacetic acid. The latter, when oxidized with chromic acid, gave scopolinic acid, 1-methyl-piperidine-2,6-dicarboxylic acid.



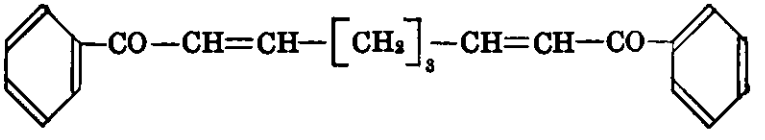
Lobelanine could be hydrolyzed to acetophenone, methylamine, and, by ring closure, fluorene or diphenyl-carbinole.



Methyl iodide treatment of lobelanine, followed by treatment with silver oxide, gave trimethylamine, a base containing nitrogen:



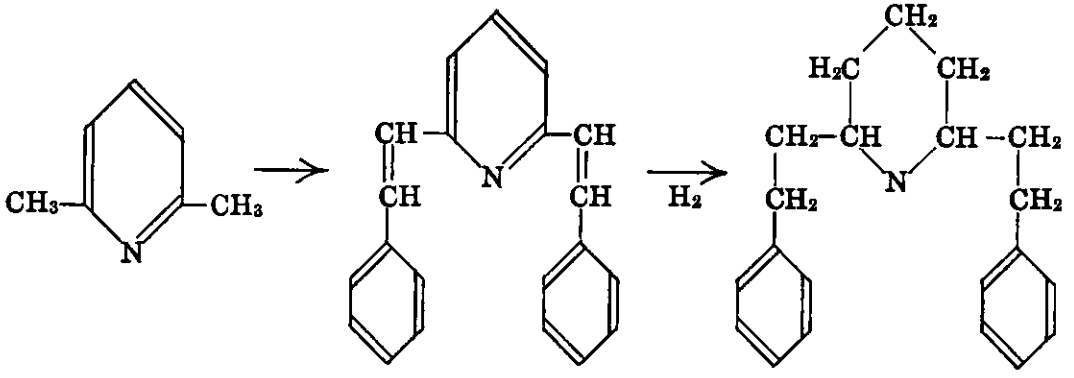
and a neutral product:



which could be reduced to either 1,7-dibenzoyl-*n*-heptane or 1,9-dihydroxy-1,9-diphenyl-*n*-nonane, depending on the type of catalyst used.[5] The nonane derivative was transformed into the heptane derivative by oxidation with chromic acid.

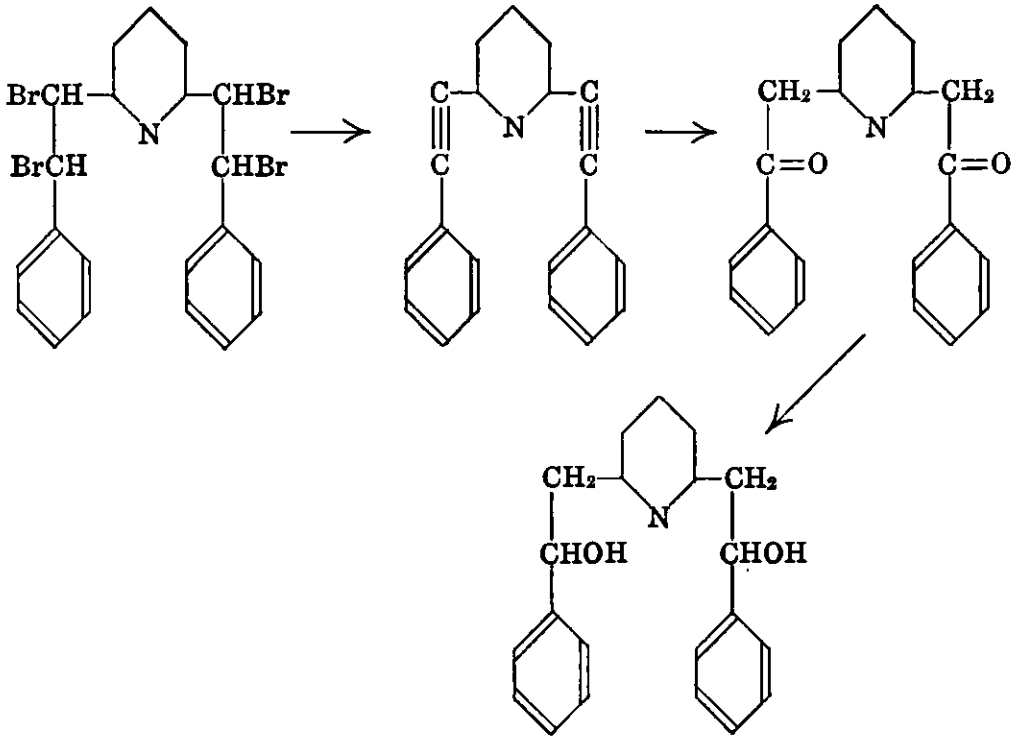
Consideration of the reactions mentioned lead to a formula for lobeline, establishing the latter as a derivative of N-methyl-piperidine with the side-chains attached adjacent to the nitrogen, in the 2 and 6 position respectively. This formula was soon confirmed by partial synthesis.

Wieland and Drishaus [6] condensed 2,6-dimethyl-pyridine with benzaldehyde according to Schuster [7] to form distyrylpyridine, which could be reduced to nor-lobelane.



That base occurred in both the meso and trans form. The meso form, which is the desired one, could be isolated by fractional crystallization of the mixed hydrochlorides. The meso base, with methyl iodide, gave lobelane methyl iodide.

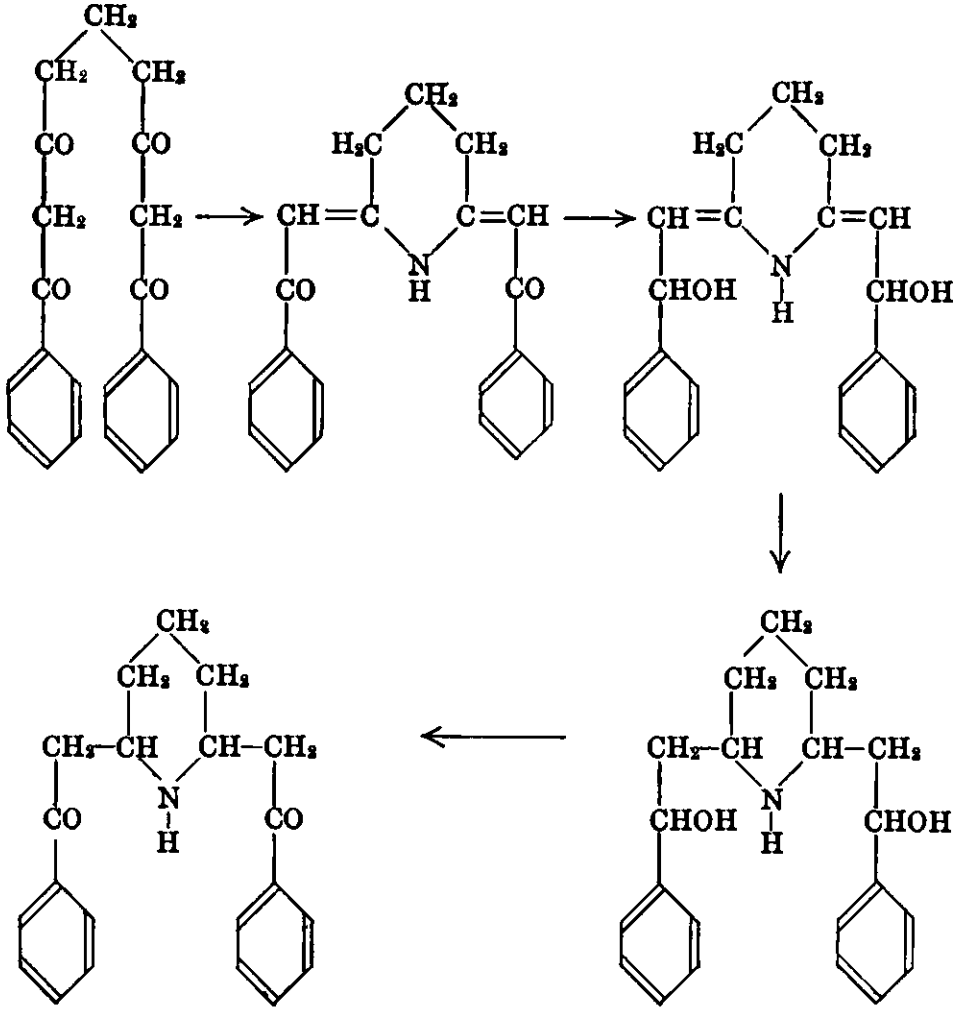
Scheuing and Winterhalder [8] brominated the distyrylpyridine mentioned above to the corresponding tetrabromide, and hydrolyzed the latter with alcoholic potassium hydroxide to 2,6-di-beta-phenyl-acetylenyl-pyridine, which on treatment with 50% sulfuric acid gave 2,6-diphenacyl-pyridine. The latter was reduced catalytically [9] to 2,6-di-(beta-hydroxy-beta-phenylethyl)-pyridine. The hydrochloride of the latter



was further reduced to the corresponding piperidine derivative, nor-lobelanidine.

By another method, the methyl-para-toluenesulfonate of the acetylenyl-compound mentioned previously was treated with sulfuric acid to form the corresponding diphenacyl compound which could be hydrogenated directly to lobelanine or lobelanidine.

Still another synthesis was performed by Wieland and Drishaus,[10] who obtained 1,7-dibenzoyl-*n*-heptane-2,6-dione by condensing methyl glutarate with acetophenone. 2,6-di-(benzoylmethylene)-piperidine was obtained by the action of dry ammonia at 100° and hydrogenated to mixtures of isomeric nor-lobelanidienes. Further reduction with aluminum amalgam followed by oxidation with chromic acid, gave nor-lobelanine.



The properties of the alkaloids of lobelia and of their salts are described in Table 12.1.

TABLE 12.1

	Alpha-Lobeline	Lobel-anine	Lobel-anidine	*d,l*-Lobeline	nor-Lobel-anine	nor-Lobel-anidine	Lobelane
Base	131	99–102	155	110–112	120–122	120	b.p. 175/1 mm
Hydrochloride	182–185	198–202	138–141	160–170	202	246	195
Nitrate	170–172	153–155	212	160	193	180	
Hydrobromide		188	190				
Methiodide			175–200			235	

Lobeline, $C_{22}H_{27}O_2N$, forms amorphous mercuri- and platinichlorides, but the other salts are crystalline. It gives a benzoyl derivative, the hydrochloride of which melts at 157°. With phosphorous trichloride it gives chlorolobelide hydrochloride, $C_{22}H_{26}ONCl \cdot HCl$ (m.p.: 174°).

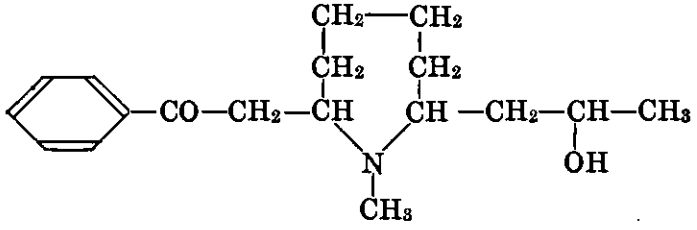
Lobelanine, $C_{22}H_{25}O_2N$, also gives an N-oxide (m.p.: 86°) besides the salts mentioned in the table.

nor-Lobelanine, $C_{21}H_{23}O_2N$,[11] has been extensively described in the literature. It can be separated from natural lobelanidine by crystallization from alcohol, whereby it will be found in the motherliquor.

Lobelanidine, $C_{22}H_{29}O_2N$, yields a dibenzoyl derivative (m.p.: 110°), and a diacetyl derivative (acetate m.p.: 75°; hydrochloride m.p.: 215°).

nor-Lobelanidine, $C_{21}H_{27}O_2N$, can be separated from natural lobelanidine as the hydrochloride because of its insolubility in boiling dry alcohol. Reduction of its methyl-para-toluenesulfonate yields lobelanidine.[12]

Another and minor alkaloid, lobinine, $C_{18}H_{27}O_2N$, occurs in lobelia in traces and has been described by Wieland *et al.*,[13] and the following probable formula has been assigned to it.



The base is not crystalline, but it yields a crystalline hydrochloride (m.p.: 144°), a hydriodide (m.p.: 130°), a platinichloride (m.p.: 190°), and a perchlorate (m.p.: 146°). A benzoyl derivative (m.p.: 147°) and an oxime (hydrochloride, m.p.: 182°) prove the presence of both a hydroxyl and a keto group.

NATURAL SOURCES

Lobelia inflata (L) (*National Formulary*, 8th Edition) is indigenous to the eastern part of the United States and Canada, where it is a common herb in neglected fields, thickets, along road sides, and in open woods and meadows. The plant is annual and has a fibrous root and an erect angular stem which averages about 1–2 ft in height. The stems are of yellow to purplish color, are quite hairy and quite branched about midway. The alternate leaves are of pale green color, oblong or ovate, and are irregularly toothed. They are up to 4 in. long. In the average drug handled in commerce, the leaves are broken and crumbled and are quite inconspicuous, but the 2-celled capsular fruits can easily be found. They are oval and striated, and the remains of the calyx can easily be distinguished. The seeds are small brown, oblong and very numerous. The many flowers are small and bunched in terminals. The corolla has a delicate blue color. When the stem of the plant is broken, a milky juice appears.

The plant is usually collected when the fruits are most numerous, in August or September. The *German Pharmacopœia* recommends collection of the plant at the end of the flowering period but before the fruits grow, and certain other *Pharmacopœiae* do not permit presence of the fruit. Sometimes the commercially available drug consists of the whole herb, while at other times the rather inert stems predominate. Careful drying of the plant in the shade is indicated to preserve the valuable alkaloids.

Lobelia has been cultivated in this country and it has been suggested to plant the seeds in the autumn.[14]

The alkaloid content of the whole plant varies from 0.2–0.7%, with the average being 0.3–0.4%. The *National Formulary* (8th Edition) does not specify a minimum content of alkaloids but restricts the amount of stems present in the crude drug to not over 10%, the amount of foreign organic matter other than stems to not over 4%, and the amount of acid-insoluble ash to not over 5%. The *National Formulary* also offers a description of unground and of powdered lobelia.

Methods for determination of the alkaloid content of lobelia have been described by Peyer and Gstirner,[15] by Caulkin,[16] by Markwell,[17] and by Lynch and Evers.[18] The method of Peyer and Gstirner, which yields good results, shall be described here in detail:

Ten grams of powdered drug are allowed to macerate for ½ hr with a mixture of 100 grams of ether and 7 grams of ammonia. Allow to macerate for at least ½ hr with frequent and thorough shaking. Filter through cotton into a 150-cc flask (a 200-cc flask was used for the initial extraction), covering the funnel with a glass plate. Expel most of the

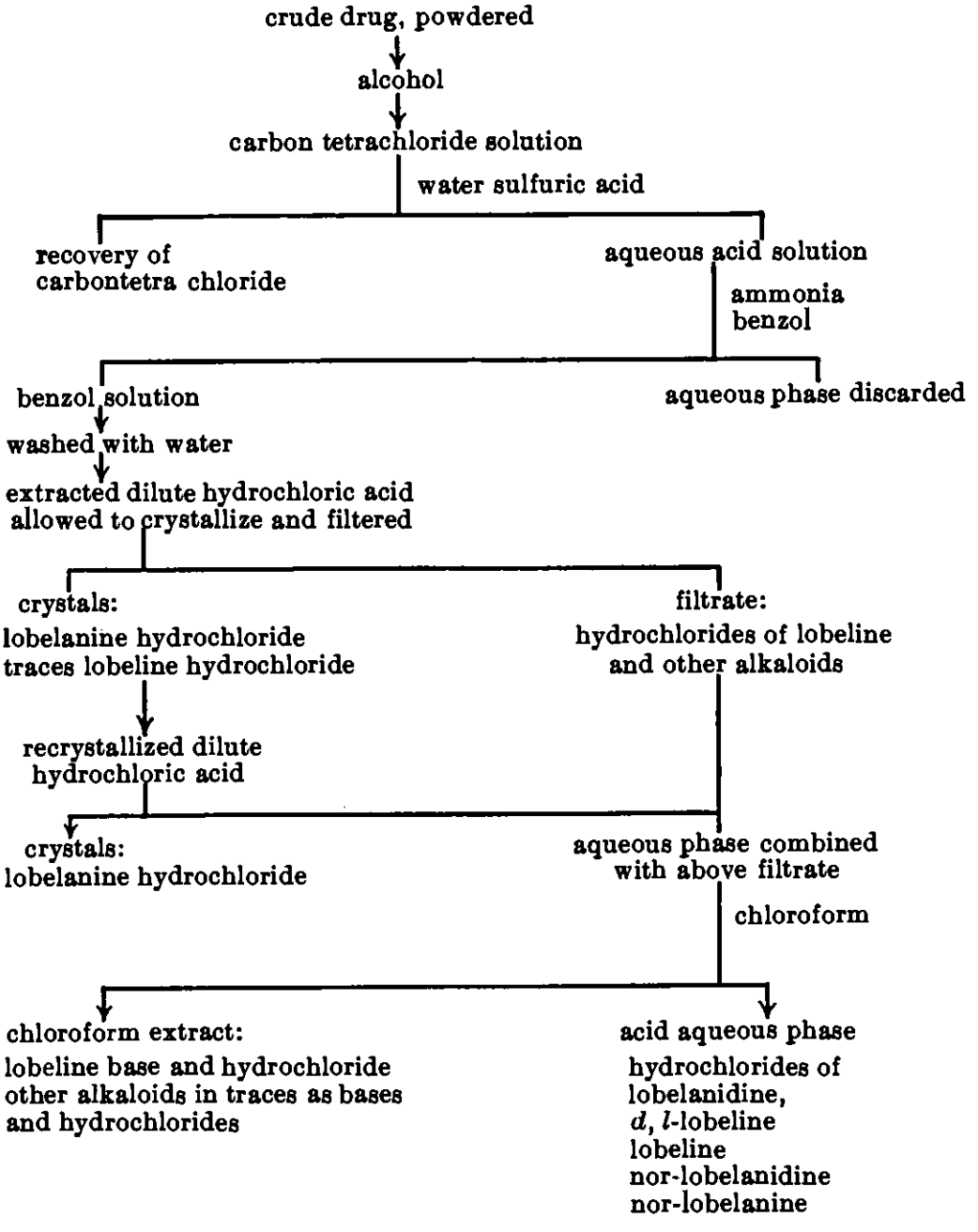
ether by adding a small amount of water to the drug. The ether is dried and 70 grams (equivalent to 7 grams of drug) is distilled to dryness, and the flask is weighed after cooling. Dissolve the residue in 10 cc of ether and add 30 cc hydrochloric acid (1 part 25% HCl and 99 parts water), mix well, and remove the ether on the steambath. Cool, bring the weight of the liquid up to 35 grams with the same dilute hydrochloric acid, and filter through a folded filter. Thirty grams of the filtrate (equivalent to 6 grams of drug) are made alkaline with dilute ammonia and are extracted thoroughly three times with 25 cc ether each time. Filter the combined ether extracts, dissolve the residue in 10 cc alcohol, add 25 cc water and three drops of methyl red, and titrate with N/10 hydrochloric acid. Each cc of the acid is equivalent to 0.0337 gram of alkaloid, calculated as lobeline.

MANUFACTURING METHODS

Good methods of manufacturing the lobelia alkaloids have been patented in Germany and in Great Britain.[19] Most of them make use of the observations that lobelanine hydrochloride will crystallize from dilute aqueous hydrochloric acid in the presence of a definite excess of that reagent, and that alpha-lobeline hydrochloride can be extracted from its acid aqueous solution with chloroform, which will dissolve a mixture of hydrochloride and base. It was found, however, that this phenomenon is not characteristic to alpha-lobeline but applies to all lobelia alkaloids, separately and even more so in mixtures.

For the manufacture of lobeline and the other lobelia alkaloids, the crude drug is reduced to a 40–60 mesh powder, which is then charged into the extractors. Two processes can be recommended, both of which have shown good results. It must be remembered, however, that the amount of "total alkaloids" varies greatly from one lot of crude drug to another. One must also bear in mind that the amount of alpha-lobeline present does not necessarily constitute an average percentage of the "total alkaloids," but that that figure often varies from 10–60%. Because of the relative ease of interconversion, all alkaloids of lobelia should be isolated and identified and should finally be transformed into alpha-lobeline.

According to the first method, the crude powdered drug is extracted four times with cold alcohol, after which a sample of the drug can be air-dried and macerated with 5% hydrochloric acid for several hours with occasional shaking. Filter the sample, make the filtrate alkaline with dilute ammonia, extract with ether, and test the ether solution for the presence of alkaloids with Mayer's reagent after extraction with dilute hydrochloric acid.



If the drug is found to be void of alkaloids, it can be drained and discarded, otherwise another extraction must be undertaken. As a rule, 3–4 extractions are sufficient.

The combined alcoholic extracts are evaporated under reduced pressure at a low temperature. At the last stages of concentration it is doubly important to keep the temperature of the batch below 45°.

It has been recommended to add 0.25–0.5% glacial acetic acid (per cent of crude drug used) or smaller amounts of tartaric or oxalic acid to the alcoholic extract prior to distillation. Whether or not this has a stabilizing influence on the lobelia alkaloids could not be deter-

mined because of the impossibility to run different distillations from one and the same lot of crude drug. It can be said, however, that the presence of small amounts of organic acids during distillation of the alcoholic extracts does not seem to hydrolyze any of the alkaloids present.

The residue from the distillation, which should not contain more than 15% of alcohol, is dissolved in three times its volume of cold ethylene dichloride, trichlorethylene, methylene dichloride, or carbon tetrachloride. This mixture should be stirred for 1–2 hr to assure solution of all the extract in the solvent. When this operation is completed, the solution is transferred to a glass-lined extractor, where it is extracted four times with dilute sulfuric acid (2–3%). After extensive stirring with time allowed for settling, all extractions should be decidedly acid to congo. The volume of the aqueous phase of the first extraction should be 3–5 times that of the solvent solution; ½–⅓ of the initial volume can be used for further extractions. After four extractions have been made, the solvent solution is usually void of alkaloids, but it should be tested, of course, before the solvent is recovered.

The aqueous acid extracts are combined, made alkaline with ammonia, and are then extracted with benzol until exhausted. Three to four extractions are usually sufficient. The total volume of benzol used should not exceed 20% of that of the aqueous phase.

The benzol extracts are combined, washed once with water, and are then extracted with small amounts of 2% hydrochloric acid. The acid should be added under continuous stirring in a glass-lined or stoneware container. The addition should take place rather slowly and under continuous testing for acidity, as too large an excess of acid cannot be recommended. The solution should be acid to congo test paper, however.

These acid extracts are combined and are allowed to crystallize overnight in the icebox. At this point, lobelanine hydrochloride will crystallize. Depending on the speed of crystallization, the crystals can either be short, stubby prisms, or long, silky needles, or both.

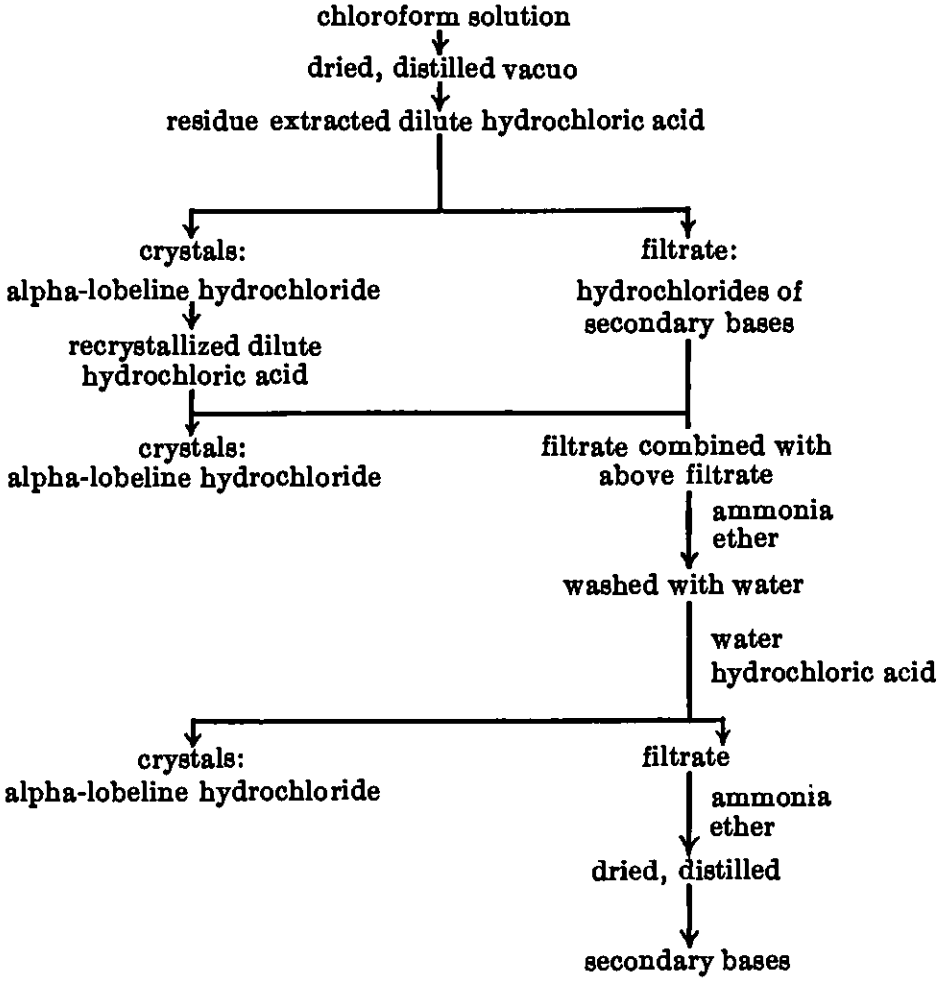
After standing overnight the crystals of lobelanine hydrochloride are filtered off and are drained well on the filter. The melting point of a thoroughly dried sample of this material should not be below 195° (m.p. of pure lobelanine hydrochloride is 202°). A low melting point indicates the presence of crystalline alpha-lobeline hydrochloride. For a separation of the two substances one or more crystallizations from N/50 hydrochloric acid is indicated. The motherliquors from these recrystallizations are combined with the original filtrate for further processing. The lobelanine hydrochloride should be stored in a vacuum desiccator or in a tight bottle in a dark place.

The combined filtrate and motherliquors containing the alpha-lobeline and traces of *d,l*-lobeline, lobelanidine are now repeatedly and thoroughly extracted with chloroform.

Ten to fifteen extractions are necessary. Each extraction is to be made with 5–10% of chloroform by volume. Thorough stirring should be continued for ½ hr or more, and about the same period of time should be allowed for settling. Extraction can be considered complete when a sample of the chloroform after drying with anhydrous sodium sulfate and evaporation leaves a negligible residue.

The chloroform extracts are combined and can be worked up according to either one of the following three procedures. The choice of method will depend on the equipment and working time available, and also on the condition and degree of contamination of the chloroform solution, which in turn depends to a certain degree upon the quality of the crude drug used.

1. The combined chloroform extracts are carefully dried with anhydrous sodium sulfate and are then distilled under vacuum at as low a



temperature as possible. The syrupy residue, which may already show signs of crystallization, is then extracted several times with five times its volume of warm water, containing a small amount of hydrochloric acid (N/100 HCl solution).

Due to the fact that the original chloroform solution contains the alpha-lobeline (and small amounts of the other alkaloids) as the hydrochloride as well as in the form of the free base, a slightly larger amount of acid will have to be added to the first aqueous extraction to compensate for the loss of hydrochloric acid suffered in the original chloroform extraction.

Extraction with warm (40–45°), dilute hydrochloric acid should be continued until no more crystals appear on cooling. The warm extracts are combined and freed from gummy impurities by straining through cheesecloth or through loose cotton. They are then allowed to crystallize in the icebox for 24 hr. Alpha-lobeline will crystallize in silky needles, which can be further purified by recrystallization from warm (40°) N/100 hydrochloric acid after treating with a very small amount of charcoal.

The motherliquors are combined, made alkaline with dilute ammonia, and are then extracted with ether until exhausted. The ether extracts are combined, washed twice with water, and are then treated with a small amount of N/50 hydrochloric acid, avoiding a large excess of acid. On cooling this, a small second crop of alpha-lobeline hydrochloride can be obtained.

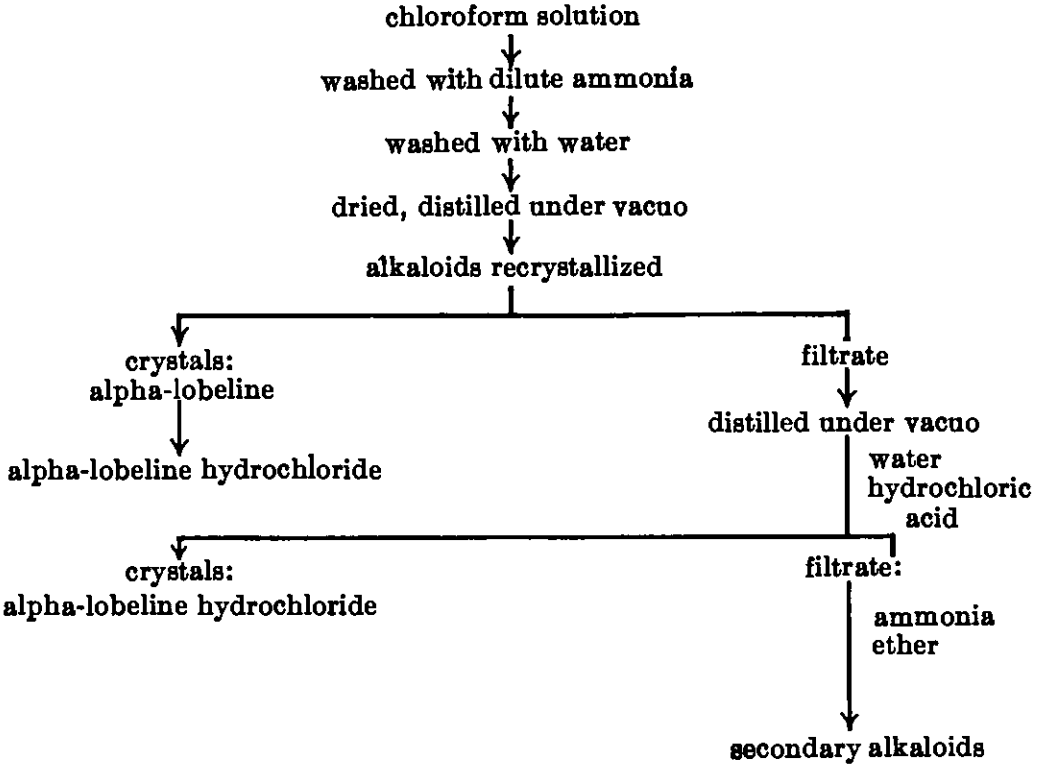
The ether from the aforementioned purification can be recovered. The aqueous acid motherliquors are made alkaline with dilute ammonia and extracted with ether. The ether extracts are combined, dried with anhydrous sodium sulfate, and are then distilled to nearly dryness. The bases thus obtained consist mostly of *d,l*-lobeline, lobelanidine, and a small amount of alpha-(*l*)-lobeline. They are combined with a similar fraction of material obtained from the chloroform extraction mentioned previously, and held for further purification and conversion.

The acid aqueous fraction remaining after exhaustive extraction with chloroform (see previous page) is made alkaline with ammonia and extracted with ether. The alkaloids remaining behind after careful drying and distillation are about the same as the ones obtained above from the motherliquors of the crystallization of alpha-lobeline hydrochloride. The bases can be recrystallized from ether-pentane, ether-isopropyl ether or from dilute alcohol. They are then ready for eventual conversion into alpha-lobeline.

2. The chloroform extracts are combined and are washed several times with dilute ammonia (2%) to remove all the hydrochloric acid

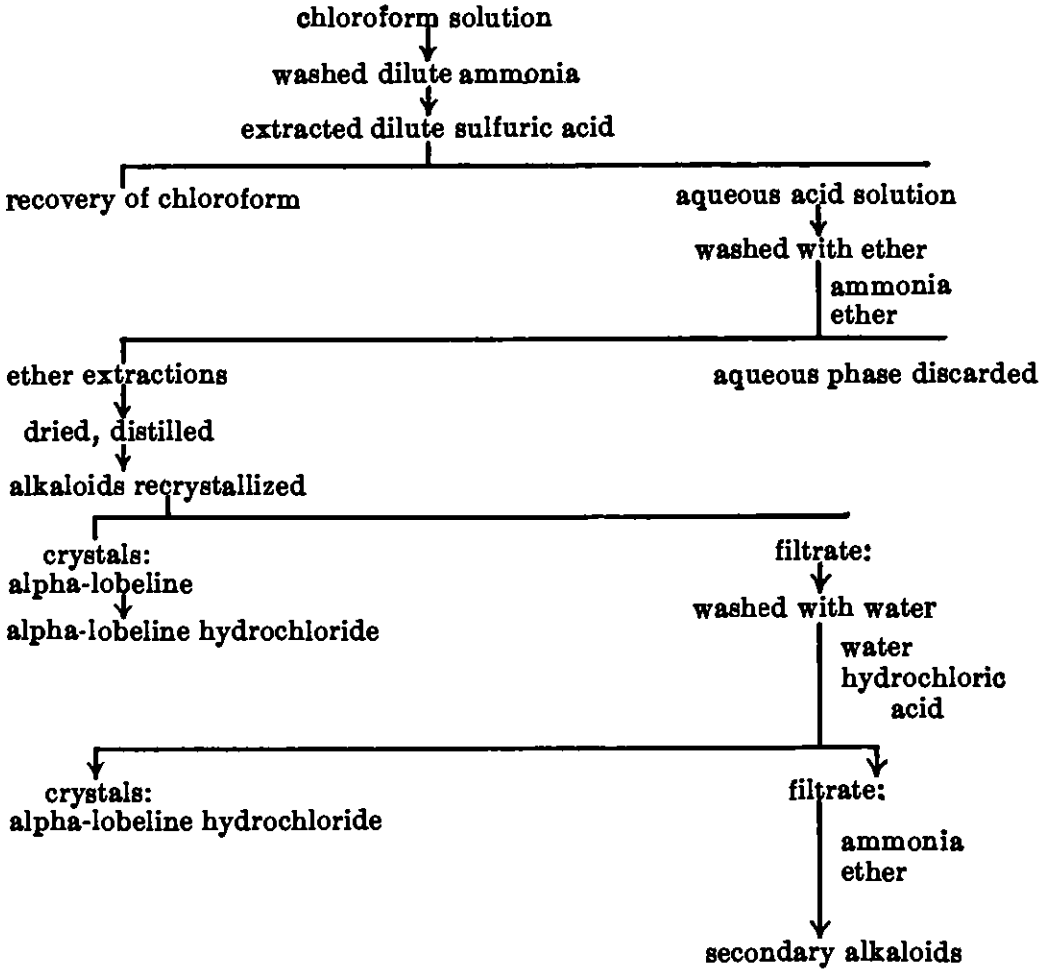
and leave the bases behind in their free state. The chloroform is then washed with water twice, dried with anhydrous sodium sulfate, and is then distilled under vacuum. The remaining bases, consisting mostly of alpha-lobeline, are recrystallized once from ether or from alcohol or benzol. The pure lobeline base is then dissolved in N/50 hydrochloric acid, and the hydrochloride can be recrystallized as mentioned before. From the motherliquors the secondary bases are recovered and purified as described before.

This second method of working up the chloroform extractions affords somewhat more of a purification than the first method. When the chloroform solution appears very discolored and impure, the following (third) method is indicated.



3. The chloroform extracts are combined and are treated with dilute ammonia and washed with water as before. They are then extracted with dilute sulfuric acid (N/50) until free of alkaloids. Three extractions should be sufficient. The aqueous acid extracts are combined and are purified once by extraction with ether. After separation the aqueous phase is made alkaline with dilute ammonia and is then extracted with ether until all the base is removed. The ether extractions are combined, dried with anhydrous sodium sulfate and distilled. The residue is recrystallized from ether, alcohol, or benzol, and alpha-lobeline hydrochloride is prepared from the pure base as described before.

Initial extraction of the crude drug can also be undertaken with dilute acetic acid (1%). Three to four extractions are necessary, and the batch should be allowed to drain well after each extraction. The clear filtrates are combined, made alkaline with dilute ammonia, and extracted with ether, isopropyl ether, or benzol. From this solvent solution the total alkaloids are extracted with dilute hydrochloric acid, and separation of the different bases is undertaken as described before if the solution is sufficiently clean. If further purification appears to be necessary, the solution is made alkaline with ammonia and extracted with ether. The ether solution either can be distilled after drying or can be extracted again with dilute hydrochloric acid. Purification and separation of the bases are then undertaken as before.



To increase the final yield of alpha-lobeline hydrochloride, the secondary bases are collected and are converted into that alkaloid. This conversion consists, in principle, of a reduction to lobelanidine and a partial oxidation to *d,l*-lobeline, followed by a resolution to obtain the laevorotatory base.

Twenty grams of mixed secondary alkaloids, consisting of lobelanidine, *d,l*-lobeline, traces of lobelanine, alpha-lobeline, nor-lobelanine, and nor-lobelanidine, are dissolved in 150 cc of pure anhydrous methanol, and the cold solution is filtered with one gram of activated charcoal. The solution is then hydrogenated at room temperature and 2–3 atmospheres pressure in the presence of Adam's catalyst. Reduction takes place rapidly and stops completely when all the keto groups have been reduced to hydroxyl groups.[20] The catalyst is then filtered off under nitrogen and the methanol is evaporated under vacuum at as low a temperature as possible. Lobelanidine is obtained in a yield of 80–90% and can be recrystallized from dilute alcohol or from ether.

Lobelanidine can be partially oxidized to a mixture of *d,l*-lobeline, lobelanidine, and lobelanine according to several methods, all of which are patented.[21]

Ten grams of lobelanidine are dissolved in 500 cc normal sulfuric acid, and 1000 cc water is added under stirring to complete solution. To this solution at room temperature is added under vigorous stirring an aqueous suspension of 2.8 grams of freshly prepared manganese dioxide. Stirring is continued for several hours, and the following day the solution is made alkaline and is immediately extracted with ether. The ether extractions are dried and evaporated to 100 cc. At this point the lobelanidine will crystallize out and can be filtered off. On further concentration of the clear filtrate the *d,l*-lobeline will crystallize. Lobelanine can be isolated as the hydrochloride from the motherliquors of the second fraction. The yield of *d,l*-lobeline according to this method is never less than 38%; the total recovery of bases is 90–95%. The *d,l*-lobeline is now ready for the resolution, the lobelanidine can be collected and used in another oxidation, and the lobelanine can be reduced to lobelanidine.

The resolution of *d,l*-lobeline with tartaric acid is also covered by patent.[21] A saturated neutral solution of *d,l*-lobeline hydrochloride at 40° is treated with the theoretical amount of neutral sodium tartrate dissolved in a small amount of water. The crude tartrate is allowed to crystallize in the cold for 24 hr, after which time it is filtered and drained well on the filter. It can be purified by recrystallizing from hot alcohol once and several times from hot water, containing small amounts of tartaric acid. The alpha-lobeline base is finally liberated and turned into the hydrochloride in the usual manner.

From the motherliquors of the resolution the *d*-lobeline can be recovered. It can be turned into lobelanine by oxidation or it can be reduced back to lobelanidine. In either case it can be used further.

Good results were also obtained when the combined secondary bases

were oxidized to lobelanine which was then partially reduced. Hydrogenation was stopped when one mole of hydrogen was adsorbed. The drawback of that method is the impossibility to obtain a uniform ratio of *d,l*-lobeline, lobelanidine, and lobelanine. Better results are obtained by using the para-toluene sulfonates instead of the free bases.

Twenty grams of the combined secondary alkaloids are dissolved in 500 cc of cold 95% acetic acid, and at room temperature a solution of 17 grams of chromic acid dissolved in 50 cc of N/10 sulfuric acid is added under stirring until a drop of the acetic acid solution placed on a piece of white filter paper shows a permanent pink coloration. The solution is then warmed on the steambath for 15 min to a temperature of 40°. The solution is then cooled, poured on ice, made alkaline with ammonia, and extracted with ether or benzol. The solvent extractions are combined, washed with water, dried with anhydrous potassium carbonate or sodium sulfate, and distilled. The residual base, consisting solely of lobelanine, can be recrystallized from dilute alcohol or from ether-pentane. To this base is added the lobelanine base obtained by suspending the crystalline lobelanine hydrochloride obtained during the process in water, liberating the base with ammonia, extracting it with ether, and drying and distilling the latter. The yield from the oxidation should be about 15 grams; the melting point of the recrystallized base should be 98–99°.

Lobelanine base or its para-toluene sulfonate are dissolved in anhydrous methanol and hydrogenated in the presence of Adam's catalyst as described before. The reduction is stopped when one mole of hydrogen has been adsorbed. The solution is evaporated under vacuum, and, if the para-toluene sulfonate has been used, the free bases are prepared and crystallized from ether. Since their ratio varies from batch to batch, no rules can be given for their separation. Fractional crystallization of the bases or their salts is employed for their separation as described later under the synthesis of lobelanine.

SYNTHESIS

It has been explained elsewhere that the synthesis of alpha-lobeline has become quite important recently due to its increased demand and the inability of meeting demands by production from the crude drug.

Two of the syntheses of lobeline are of commercial importance. One starts from 2,6-dimethylpyridine while the other starts from glutardialdehyde.

The synthesis starting from lutidine [2,6] is mostly based on the report of Scheuing and Winterhalder.[20]

250 grams of lutidine are heated with the theoretical amount of benzaldehyde (495 grams) and 75 grams of anhydrous zinc chloride in an autoclave at 210° for 2–3 hr or are refiuxed. The autoclave is then cooled rapidly, and the mass of crystals is recrystallized from anhydrous methanol. The yield depends to a great extent upon the purity of the lutidine used and varies from 75–90% (m.p.: 165–170°).

The resulting 2,6-distyrylpyridine is dissolved in five times its weight of carbon tetrachloride or ether, and the theoretical amount of bromine dissolved in the same solvent is added to form the tetrabromide. The addition is undertaken at room temperature and under constant stirring. The tetrabromide crystallizes out and can be filtered off. The yield in this step is 90% of theory or more (m.p.: 180–185°).

This tetrabromide is then treated with a 50% excess of 15% alcoholic potassium hydroxide (500 cc solution per 100 grams material). The mixture is boiled under continuous stirring until most of the alcohol has distilled. The residual mass containing 2,6-di-(beta-phenylethinyl)-pyridine is then filtered and washed with water to remove potassium bromide and excess potassium hydroxide.

The ethinyl compound is now dissolved in 2–4 times its weight of benzol and is treated with an equal weight of methyl-*p*-toluene sulfonate or the corresponding benzene compound. The reaction is at first exothermic, but it soon subsides and it is necessary near the end to apply heat for at least ½ hr to bring the reaction to completion. The quaternary salt of the di-ethinyl compound crystallizes on cooling and is filtered off and dried.

This compound is transformed into 2,6-diphenacyl pyridine by heating for 15 min in 85% sulfuric acid (280 cc acid per 100 grams compound) to near boiling. This reaction is at first quite violent. Upon adding the cooled solution to a large amount of cold water the quaternary salt of 2,6-diphenacyl pyridine is precipitated. It is filtered and washed with water and finally recrystallized from ethanol or from methanol.

Reduction of this compound is carried out in methanol (15–20% solution) with hydrogen (5 atm), in the presence of Adam's platinum oxide catalyst (1–2%) at room temperature. Adsorption of one mole of hydrogen takes place rapidly, after which time the hydrogenation is stopped and the batch is filtered under nitrogen. The methanol is evaporated under vacuum, and the residue consists of a mixture of lobelanine, lobelanidine and *d,l*-lobeline, the proportions of which are unpredictable. Separation of the bases must therefore vary from batch to batch, and no set rules can be given. Fractional crystallization from ether-isopropyl ether or from ether-pentane is often effective. Another possibility is by way of different salts of different solubility. The hydro-

chloride of lobelanine is slightly soluble in dilute hydrochloric acid (0.3% in a 5% hydrochloric acid solution at 10°), whereas the nitrate of lobelanidine is practically insoluble in acetone when it is in a fairly pure state. Other salts which have been investigated are the salicylates, the phosphates, and the hydrobromides.

Alpha-lobeline can finally be obtained from its racemate by crystallization of the tartrate or the dibenzoyl-tartrate. The over-all yield of the hydrochloride, based on lutidine, varies from 7–15%.

The preparation of lobelanine from glutardialdehyde is based mainly on the work originated by Schoepf and Lehmann [22] and consists of condensing glutardialdehyde, methylamine and benzoylacetic acid in a buffered solution. This reaction is simple and efficient and its only drawback is the difficulty of obtaining the glutardialdehyde.

This latter compound has been prepared from cyclopentene, from trans-cyclopentanediol-1,2, from trimethylene-bromide, and from pyridine.

To manufacture glutardialdehyde from trimethylenebromide, 1130 grams sodium are dissolved in 8000 cc of absolute alcohol and the solution is placed in a 24-liter flask equipped with stirrer and reflux. To this is added a mixture of 5000 grams of trimethylene bromide and 8000 grams malonic ester as fast as possible, at such a rate as to prevent the contents of the flask from boiling. When the addition is complete, the mixture is heated under stirring until a sample of the batch upon dilution with water does not show a very distinct alkaline reaction. When the reaction is complete the batch is dumped on ice, extracted with benzol three times, and the benzol distilled without drying until the residue in the flask reaches a temperature of 85°. The residue is then subjected to a steam distillation to remove excess of trimethylene bromide and malonic ester and some tetramethylene dicarboxylic ester formed during the reaction. The residue from the steam distillation is then dissolved in a small amount of ether, dried, and distilled under vacuum. Pentane-tetracarboxylic ester boils at 210–215°/12 mm Hg or at 233–236°/30 mm Hg. The yield varies from 3200–3400 grams.[23]

3400 grams of the tetracarboxylic ester are heated on the steambath for two hours with 3160 cc of hydrazine hydrate (85%) and 3000 grams of isopropyl alcohol (98%). The tetrahydrazid will start to crystallize from the hot solution and can be filtered off after the solution has cooled for several hours. It usually is quite pure but can be recrystallized from hot water on addition of isopropyl alcohol. The melting point is 202° and the yield 1700–2000 grams or higher.

This tetrahydrazid is dissolved in the theoretical amount of cold, dilute (5%) hydrochloric acid, mixed with a sufficient amount of ether,

and the mixture is cooled to below 0° under rapid stirring. A closed container equipped with a reflux condenser might be advisable to keep the loss of ether to a minimum. The theoretical amount of a cold, dilute solution of sodium nitrite is then added slowly, keeping the temperature at 0°. When the diazotization is finished, all the azide formed should be in the ether layer. The latter is separated and the aqueous phase is washed once with ether. The ether extractions are combined and are dried thoroughly with three successive portions of sodium sulfate. An equal volume of dry absolute ethanol is then added, the ether is distilled off, and the alcohol solution is refluxed under protection from moisture until the evolution of nitrogen has ceased. Most of the alcohol is then distilled off, and the diurethane is allowed to crystallize. This urethane, upon treatment with the theoretical amount of warm, 2 N sulfuric acid, gives a quantitative yield of glutardialdehyde, which can be purified by steam distillation. The amount can be determined quantitatively by treating a sample of the solution with an excess of para-nitrophenylhydrazine in N/10 hydrochloric acid, filtering, washing and weighing the precipitate when dry.

Another method of manufacturing glutardialdehyde consists in making glutardioxime from 1,4-dihydropyridine and diazotizing the former to the free dialdehyde.[24] One pound of dry pyridine is dissolved in 1 gal of dry alcohol, and 4.75 oz of sodium are added to the boiling mixture. The pyridine must be at least 98–99% pure; the alcohol must also be anhydrous and can be either methanol, ethanol, isopropanol, or butanol. When all the sodium has reacted, 8.5 oz of hydroxylamine hydrochloride are added fast, but in small portions and under constant stirring, permitting the ammonia which is liberated to escape. As soon as this reaction is nearly complete, the solution is carefully neutralized by the addition of concentrated hydrochloric acid, and refluxing is continued under stirring for 2–3 hr. The solution is then filtered hot, the alcohol is removed under vacuum, and the residue is recrystallized from water. The yield of glutardioxime varies from 4.5–6 oz.

The free dialdehyde can be prepared by suspending the dioxime in dioxane and treating it under stirring and cooling with the theoretical amount of ethyl nitrite, either as such or in the presence of glacial acetic acid. When the evolution of nitrogen has subsided, the solution is allowed to come to room temperature. It is then diluted with water and it can be used as such, after its content of glutardialdehyde has been determined.

A better method consists in suspending the dioxime in the theoretical amount of normal sulfuric acid or hydrochloric acid, and treating with an aqueous solution of sodium nitrite. In this way, glutardialdehyde

is obtained from its dioxime quantitatively in an aqueous solution which can be used immediately.

To prepare glutardialdehyde from cyclopentene the latter was dissolved in acetic ester and treated with ozone.[25] That oxidation product was not isolated but was immediately hydrogenated in the presence of palladium on calcium carbonate until the hydrogenation stops. The mixture is then fractionated under vacuo and the resulting glutardialdehyde is immediately dissolved in water.

According to still another method, trans-cyclopentanediol-1,2 is dissolved in dioxane and is then oxidized with lead tetra-acetate. After the lead acetate formed during the reaction is filtered off, the filtrate is fractionated under vacuo. Glutardialdehyde distils at 74–78°/16 mm Hg and is immediately dissolved in water.[22]

Benzoylacetic acid is usually made from ethyl benzoyl acetate, the synthesis of which has been described.[26] Ethylacetoacetate dissolved in benzene is reacted with sodium and then with benzoyl chloride to form ethyl-benzoylacetoacetate which is then hydrolyzed to ethyl benzoyl acetate. This synthesis has also been performed starting with ethyl-benzoate and ethyl-acetoacetate. The benzoylacetic ester should be stored in the icebox, and should be freshly distilled before use (b.p.: 165–170°/20 mm Hg).

The free acid is prepared from the ester by dissolving the latter in a 5–10% solution of sodium hydroxide containing 1.25–1.5 moles of alkali for each mole of ester and permitting the solution to stand overnight. This solution is carefully neutralized to litmus with hydrochloric acid just before using. The temperature should never be allowed to rise above 25°. Ice can be introduced into the solution directly during the process of neutralization. The final solution contains 85% of the theoretical amount of benzoylacetic acid.

To effect the condensation to lobelanine, the following solutions are introduced in the order named and the mixture is allowed to stand in the light for at least 24 hr. The temperature should at no time be above 25° nor below 20°. To the neutral or slightly acid solution of 420 grams of benzoylacetic acid is added rapidly a solution of 635 grams of citric acid and 243 grams of sodium hydroxide, an aqueous solution of 90 grams of glutardialdehyde, a solution of 71 grams of methylamine hydrochloride and 180 cc of concentrated hydrochloric acid, the whole batch to be made up to a volume of 20 liters. At this point the pH of the solution should be about 4. If this is not the case, more hydrochloric acid must be added. Evolution of carbon dioxide will start within 1–2 hr. The batch should be mixed occasionally. After 12 hr standing, an additional 100 cc concentrated hydrochloric acid are added.

After standing for 24 hr at room temperature, the batch is thoroughly chilled for 4 hr, and the crystals of lobelanine hydrochloride are then filtered off. A further quantity of concentrated hydrochloric acid (100 cc) is added to the motherliquor, which is allowed to stand for an additional 24 hr at room temperature. If the second crop of lobelanine hydrochloride is considerable, the crystals can be filtered off, otherwise the batch should be discarded. A small amount of lobelanine can be recovered from the liquor by making the latter alkaline with ammonia and extracting it several times with benzol. The benzol extractions are combined, washed with water, and are then extracted with dilute sulfuric acid several times. The aqueous acid extractions are combined, washed once with ether, are then made alkaline, and extracted with ether. Lobelanine can finally be recovered from the ether by extraction with dilute hydrochloric acid, but the amount is usually so small that it is hardly worth the trouble. The total yield of crude lobelanine hydrochloride is usually from 180–230 grams dry.

The crude lobelanine hydrochloride is suspended in water and is washed with ether twice. The base is then liberated by the addition of ammonia and is finally extracted with ether or with benzol. After drying and distilling the solvent, the lobelanine base can be crystallized from dilute alcohol or from ether-pentane.

Lobelanine is then converted into *d,l*-lobeline by partial reduction or by complete reduction followed by partial oxidation as described before.

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

Lobelia leaves, when chewed raw or otherwise administered, cause headaches, nausea, and vomiting, followed by sweating and a general relaxation of the whole body. The pulse becomes rapid and feeble, the voluntary motion of body muscles fails, and, finally, if the dose was toxic, coma sets in. Death is sometimes preceded by convulsions. Occasionally the vomiting is not strong enough to remove all the alkaloids, in which case the toxic symptoms are more severe.

It has been said that pure alpha-lobeline does not give the emetic and other secondary effects which the mixture of lobelia alkaloids exhibits. The pharmacological actions of lobeline resemble those of nicotine and are quite complex.[27] After a preliminary phase of excitation and stimulation, it paralyzes the involuntary nervous system. The sympathetic and parasympathetic nervous systems also show a certain sensitivity toward the alkaloid, and its influence upon the body functions depends upon the relative sensitivity of those systems.

Lobeline has been recommended for use as an emergency respiratory stimulant, but it was found that the efficient and reliable dose is too close for comfort to the lethal dose.[28] It is for that reason, although it is thought to be effective, that it is too dangerous to use for the resuscitation of newborn infants.[29] In all cases the effects are transient, but it deranges the digestion.

Administration of alpha-lobeline is followed by changes in the pulse rate and the blood pressure,[80] the latter being lowered with large doses. It has been used in spasmodic bronchitis, chronic emphysema, and spasmodic croup as a nauseating expectorant, being administered between the paroxysms, which may occasionally be stopped completely by large doses.

Intramuscular injection in animals is followed by increased respiration, raised blood pressure, and a slowed heart beat. Intravenous injection also causes intestinal contraction. In large doses the respiratory depression of morphine, and to a lesser degree of aliphatic narcotics, is overcome for a short period of time, but, as the stimulating effect of lobeline wears off, its depressing influence is added to that of morphine.

Oral administration of alpha-lobeline has been recommended to break the smoking habit.

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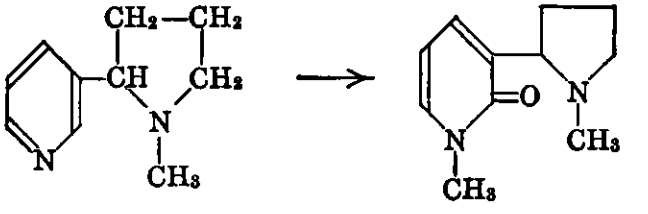
CHAPTER XIII

NICOTINE

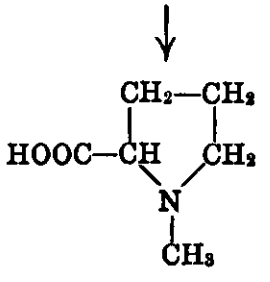
CHEMISTRY

Nicotine is the main toxic principle in tobacco, which comprises different species of Nicotiana. It is accompanied by a large number of secondary alkaloids, most of which are found in all types of tobacco[1]. Through these secondary alkaloids, interesting connections have been found with plants belonging to species other than Nicotiana, for instance Duboisia (Solanaceae), Anabasis (Chenopodiaceae) and Lupinus (Leguminosae).[2] Simpler bases, such as ammonia, trimethylamine, isoamylamine, piperidine and 2,3′-dipyridyl, have also been isolated.[3]

Nicotine, $C_{10}H_{14}N_2$ (b.p.: 246.1/730.5 mm, α_D −168.4 to −168.66°), is soluble in alcohol, ether, light kerosene, and in water below 60° and above 210°. Between those two temperatures the solubility of nicotine in water is limited.[4] The salts are easily soluble in water, where they are dextrorotatory. Aqueous solutions of the hydrochloride and sulfate, when autoclaved to 250°, become racemized.[5] The base can be purified either by vacuum distillation, preferably under an inert atmosphere, or by distillation with steam.



N-Methyl-nicotone



Hygric acid

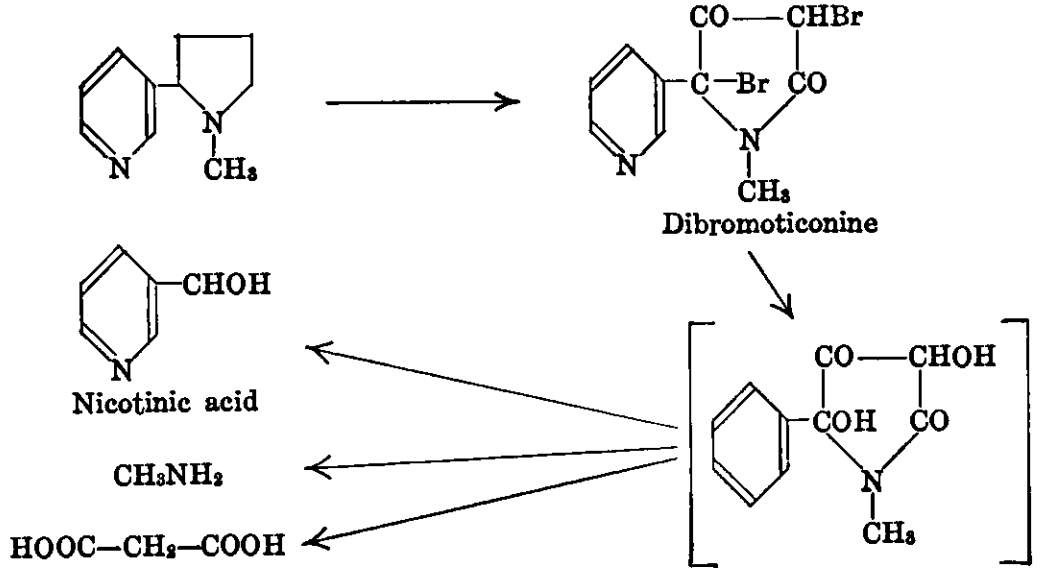
Nicotine can be oxidized to nicotinic acid, and, if nitric acid is used, 3-nitro-5-(3′-pyridyl)-pyrazole,[6a] but if nicotine-iso-methiodide is oxidized, N-methyl-nicotone is obtained.[6b]

N-methyl-nicotone can be oxidized to *l*-hygric acid, whose optical configuration can be correlated to that of *l*-proline and *l*-stachydrine. Strangely, the optical configuration of *d*-nor-nicotine, found in *Duboisia hopwoodii*, is opposite of that of nicotine and proline.

Reduction of nicotine with sodium and fusel oil yields hexahydronicotine,[7] and distillation of the zinci-chloride of nicotine with lime furnishes pyridine, pyrrole, and methylamine.[8]

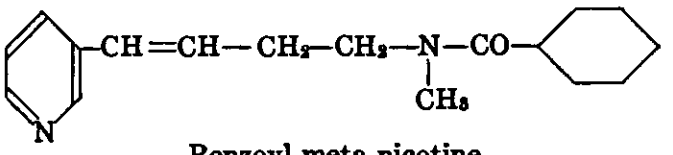
When nicotine is dissolved in glacial acetic acid and is brominated in that solution, the product which is obtained is a hydrobromide perbromide, $C_{10}H_{10}ON_2Br_2 \cdot HBr \cdot Br_2$, from which dibromocotinine can be obtained, $C_{10}H_{10}ON_2Br_2$.[9] This compound gives no ketone or hydroxyl reaction, but it can be degraded to 3-acetylpyridine, oxalic acid, and methylamine.

When the nicotine is dissolved in hydrobromic acid prior to bromination, dibromoticonine, $C_{10}H_8Br_2O_2N_2$ is obtained. Under varying conditions, this can be reduced to methylamine, and gamma-pyridyl-beta, gamma-dihydroxybutyric acid, or, from the latter, malonic and nicotinic acids.



Under different conditions, the pyrrolidine ring is opened to furnish meta-nicotine (iso-nicotine) and its derivatives. Benzoylation leads to the formation of benzoyl-meta-nicotine;[10] hydrogenation yields octahydro-meta-nicotine.[11]

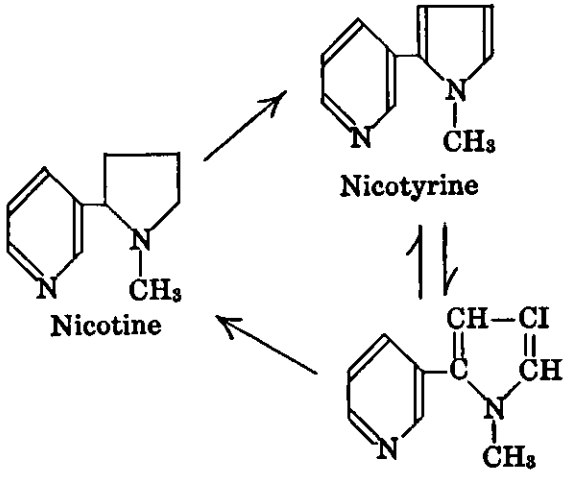
When nicotine hydriodide is treated with methyl iodide, nicotine-iso-methiodide is obtained, which can be oxidized to trigonelline.[12] The



Benzoyl-meta-nicotine

constitution of meta-nicotine has been confirmed by a series of reactions, leading to *d,l*-nicotine.[13] Convincing arguments in favor of the formula of nicotine are the facts that alkaloids contain no =NH group but have one N-methyl group which is not attached to the pyridine nucleus.

Mild oxidation or dehydrogenation of nicotine leads to the formation of nicotyrine, $C_{10}H_{10}N_2$,[14] which has also been obtained from tobacco leaves.[15] It has been reduced to nicotine through iodonicotyrine,[16] and it is an intermediate in Pictet's synthesis of the latter.[17]



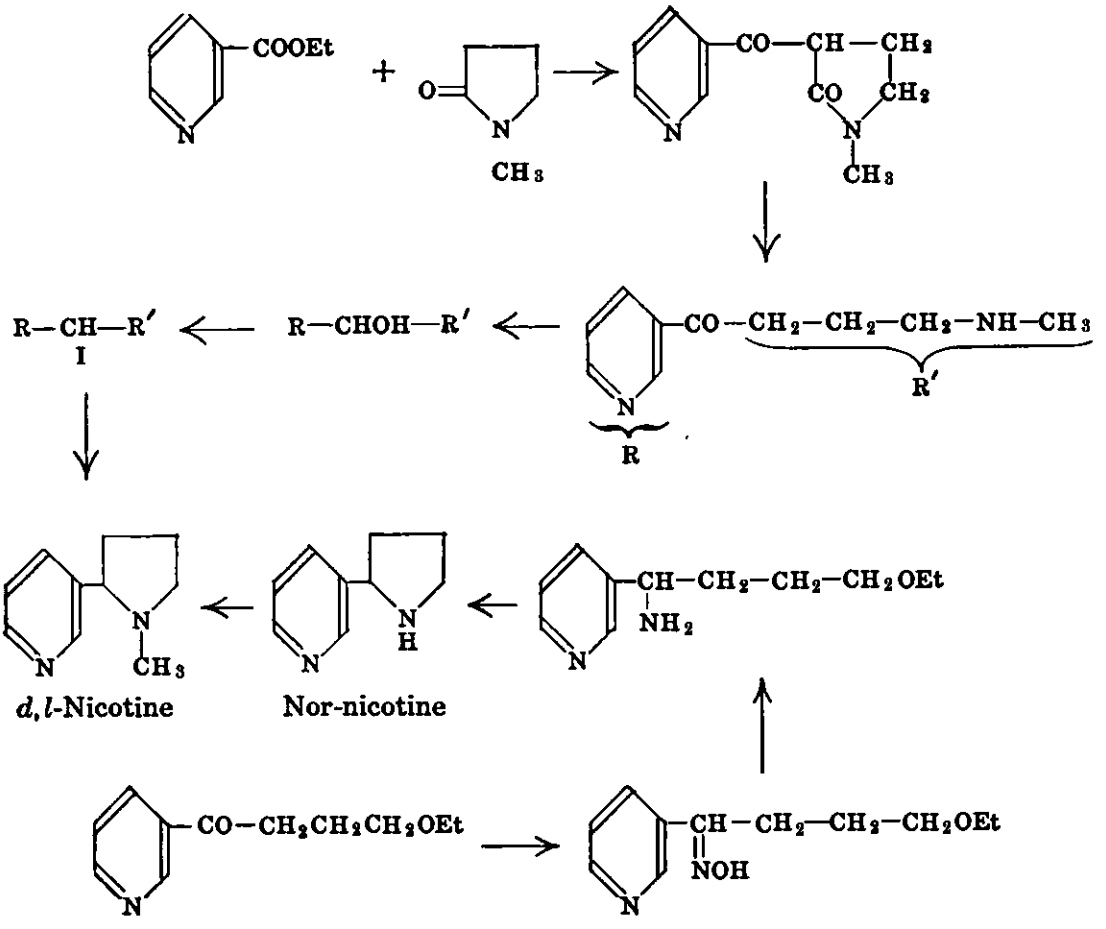
Nicotyrine

Nicotine

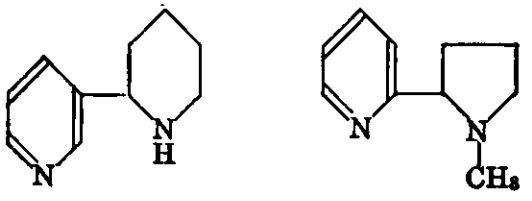
Iodonicotyrine

Partial reduction of nicotyrine leads to the formation of 4,5-dihydronicotyrine,[18] which can be reduced further to nicotine, nicotyrine, and dihydro-meta-nicotine, the first two being formed by disproportionation of hydrogen. Nicotyrine has been reduced to *d,l*-nicotine, the latter can also be called tetrahydro-nicotyrine, and the racemic base has been resolved through the ditartrate.[19] Syntheses of racemic nicotine have been effected by Spaeth and Bretschneider[20] and by Craig.[21] The former starts from ethyl nicotinate and 1-methyl-pyrrolidone, while the latter starts from nicotinic acid nitrile and ethyl-gamma-bromomagnesiumpropyl ether.

Two isomers of nicotine are known: one is a synthetic compound whereas the other accompanies natural nicotine. The latter, nicotimine, $C_{10}H_{14}N_2$, has been isolated as the nitrosamine,[22] and the base has been purified by distillation of its benzoyl derivative. Its salts have been



described by Pictet and Rotschy.[22] At least two alpha-nicotines, differing slightly in their physical constants, have been synthesized.[23] They differ from nicotine by having the pyridine nucleus substituted in the 2- instead of the 3-position (1-methyl-2-(2′-pyridyl)-pyrrolidine).



Nicotimine α-Nicotine

nor-Nicotine, $C_9H_{12}N_2$, has been prepared by treating nicotine-N-oxide with acetic anhydride [24] and hydrolyzing the resulting N-acetyl-derivative. It has also been prepared from nicotine by a different method, together with meta-nicotine.[25] Its laevo-form has been isolated from tobacco,[26] but its dextro-form is found in *Duboisia hopwoodii*.[27] *l*-nor-Nicotine has been converted to *l*-nicotine by methylation, and the latter, on careful oxidation, yields *l*-nor-nicotine again.[27]

NATURAL SOURCES

Nicotine is usually made from the waste tobacco which accumulates from the manufacture of cigars, cigarettes, and pipe tobacco.

Tobacco consists of the dried leaves of *Nicotiana tabacum* or *Nicotiana rustica*, and both are cultivated in North America on a large scale. Other species are grown in different parts of the world. Little need be said here concerning the highly specialized science of growing and curing tobacco. Its nicotine content is reduced if the plants are grown in the shade or in wet soil; it increases with higher temperature, and nitrogen-rich fertilizers. The plant reaches its maximum content of nicotine when it is fully ripened; its seeds are usually void of that alkaloid. Treatment of the seeds with a solution of colchicine prior to planting may increase the nicotine content of the plant by as much as 138%.[28]

Intensive investigations have been undertaken to determine the alkaloid contents of different species,[29] the points of production of the alkaloid in the plant,[30] and the influence of grafting and cultivating upon the inherited capabilities of nicotine-production of plants.[31]

MANUFACTURING METHODS

Nicotine is produced from tobacco waste on a large scale, and it is obtained in commerce in aqueous solution, either as the sulfate or the oleinate, containing definite amounts of nicotine per volume. The product is semicrude but is sufficiently pure for its use as a horticultural insecticide.

The alkaloid is liberated from the tobacco by treatment with lime, and it is then removed either by steam distillation or by extraction with a solvent such as kerosene.

Steam distillation is more economical and easier to handle on a large scale. Its difficulties are incomplete removal of nicotine due to channeling, and decomposition of a certain percentage of the alkaloid and of other alkaloids due to the prolonged action of alkali and of steam. This latter difficulty has been overcome by mixing the powdered tobacco waste with water and a solution of lime to a thin paste, and pouring that down a series of columns similar to those which are used to remove the last traces of alcohol from fermenting mesh. The steam moves countercurrent and removes ammonia and volatile alkaloids. It is somewhat cooled and is finally condensed in dilute sulfuric acid. A patent has been granted [32] according to which the nicotine is adsorbed from the steam on activated charcoal from which it is later removed (eluted) by means of a solvent. If the tobacco, after treating with

alkali, is extracted with kerosene or another solvent, that solution is extracted with dilute sulfuric acid after separation from the tobacco. A number of patents have been issued for processes of denicotinization of tobacco,[33] or for the manufacture of nicotine.[34]

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

Nicotine, when administered to the body, initially causes stimulation which is later followed by depression and paralysis of the autonomic ganglia. Although it has been used occasionally in medicine to relieve some of the symptoms of post-encephalitic parkinsonism,[35] its sole use at this time is as a horticultural insecticide.

ANALYTICAL METHODS

Nicotine yields precipitations with various reagents from very dilute solutions: platinic chloride (1:5000), gold chloride (1:10,000), Mayer's reagent (1:15,000), bismuth potassium iodide (1:40,000) and iodine-potassium iodide (1:250,000). A characteristic color is obtained with para-dimethylaminobenzaldehyde.[36]

Because of the importance of the quantitative estimation of nicotine, a survey of the appropriate literature is given.[37]

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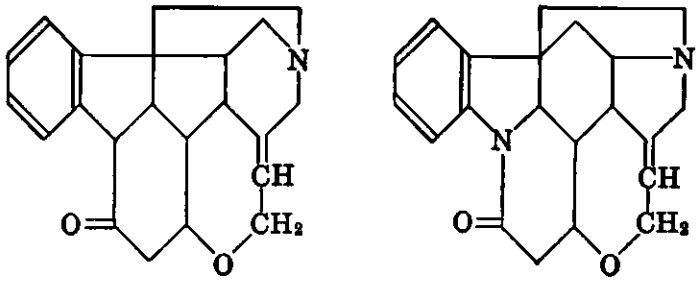
CHAPTER XIV

THE ALKALOIDS OF NUX VOMICA

CHEMISTRY

Strychnine is the commercially most important alkaloid obtained from this Strychnos species and from *Strychnos ignatii* Berg. In both drugs it is accompanied by an approximately equal amount of brucine. Other alkaloids are also found with these two bases and accumulate in the motherliquors of their commercial preparation. They are, however, of no importance, and they will be mentioned only briefly at a later place.

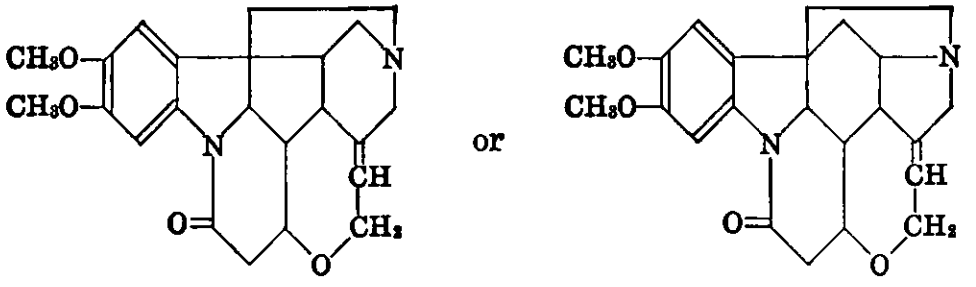
The structure of strychnine or brucine has not been definitely determined as yet, although the following formulae suggested by Prelog and Szpilfogel [1] and by Robinson [2] are probably accurate with the possible exception of minor details.



Determination of the structure of strychnine and brucine has proved difficult because of the compactness of the molecule and its relative resistance to degradation, due to their lack of points vulnerable to attack. Because of the voluminous nature of the literature concerning the chemistry of these alkaloids, the following summary is intended solely as a survey. As many references as possible have been included, and the reader should refer to the original literature for details.

Strychnine and brucine seem to be closely related. The difference, as indicated by their respective empirical formulae, is probably due to the absence of two methoxyl groups in strychnine. However, the degradative conversion of brucine to strychnine has not been accom-

plished as yet. Partly because of the formation of bis-apo-methylbrucine, the methoxyl groups are thought to be attached to an aromatic nucleus. Formation of substitution products also points toward the presence of an aromatic nucleus. The methoxyl groups are probably ortho to each other, as shown in the following partial formula of brucine:[3]



Reduction Products of Strychnine

The reduction of strychnine yields a number of different compounds under varying conditions.[4]

Hydriodic acid and phosphorus yield desoxystrychnine, $C_{21}H_{26}ON_2$, $3H_2O$ (m.p.: 75°, 172° anhydr.), which gives rise to desoxystrychninic acid with sodium methoxide in methanol. The lactame group is thought to be unchanged in the former compound. Further reduction of desoxystrychnine with sodium and amyl alcohol yields strychnoline,[5] $C_{21}H_{26}N_2$, but electrolytic reduction yields dihydrostrychnoline, $C_{21}H_{28}N_2$:

$$C_{20}H_{22}ON\begin{cases}-CO\\ |\\ =N\end{cases} \rightarrow C_{20}H_{26}N\begin{cases}-CO\\ |\\ =N\end{cases} \rightarrow C_{20}H_{24}N\begin{cases}-CH_2\\ |\\ =N\end{cases}$$

Strychnine — Desoxystrychnine — Strychnoline

Electrolytic reduction of strychnine yields strychnidine,[6] $C_{21}H_{24}ON_2$ (m.p.: 251°). According to Zwicker and Robinson,[7] the reduction is thought to take place by way of tetrahydrostrychnine:

$$C_{20}H_{22}ON\begin{cases}-CO\\ |\\ =N\end{cases} \rightarrow C_{20}H_{22}ON\begin{cases}-CH_2OH\\ |\\ =NH\end{cases} \rightarrow C_{20}H_{22}ON\begin{cases}-CH_2\\ |\\ =N\end{cases}$$

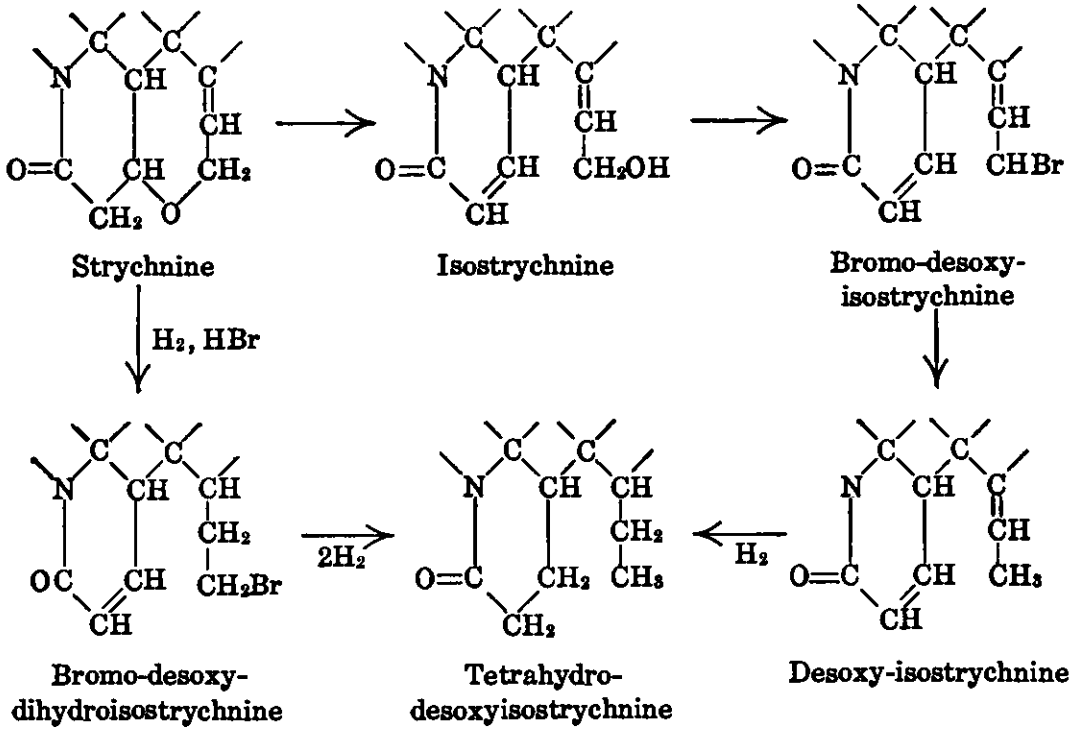
Strychnine — Tetrahydrostrychnine — Strychnidine

Strychnidine resembles methyltetrahydroquinoline in its color reaction. In both, tetrahydrostrychnine and strychnidine, both nitrogen atoms are basic, and two series of salts can be obtained.

Reduction of strychnine with Raney-nickel at 220° and 150 atm[8] gives rise to a base, $C_{21}H_{26}O_2N_2$ (m.p.: 252°), a base of m.p.: 190°, some dihydrostrychnine, trimethylamine, and an unidentified neutral

product. The compound m.p.: 252° gives the strychnine color reactions, and upon electrolytic reduction it yields a base of the strychnidine type. Although one ring seems to have been opened, ring D is assume to have remained intact. The other nitrogen, $N_{(b)}$, was found to be tertiary as in strychnine and does not yield a nitroso-derivative. There is no alkoxy group present, but the compound gives an acetyl-derivative. The oxygen-containing ring E was thought to have opened, giving rise to a primary or secondary alcohol.

The reduction of strychnine to dihydrostrychnine and pseudostrychnine has recently been discussed by Leuchs,[9] and the conversion of dihydrostrychnidine D into dihydrostrychnidine A has been discussed by Achmatowicz and Robinson,[10] who also discuss the possible structure of strychnine. The reduction of chlorostrychnine has been described by Leuchs and Steinhorn,[11] and the reduction of bromo-desoxy-isostrychnine and bromo-desoxy-dihydroisostrychnine has been described by Leuchs and Schulte.[12] The latter authors depict the formation of tetrahydro-desoxyiso-strychnine as follows:



The presence of an ethylenic linkage is indicated by the fact that strychnine can be converted into hexahydrostrychnine either by way of the dihydro- or the tetrahydro-derivative, but dihydrostrychnine yields also dihydrostrychnidine A, which in turn can be obtained from hexahydrostrychnine by dehydrogenation.

The distinction between the two nitrogen atoms of strychnine and brucine has been briefly indicated above. Only one of them shows basic properties, and it is conveniently called $N_{(b)}$, whereas the other, showing no basic property, is part of a cyclic amide configuration. This group can be changed as follows:

$$=N—CO—$$

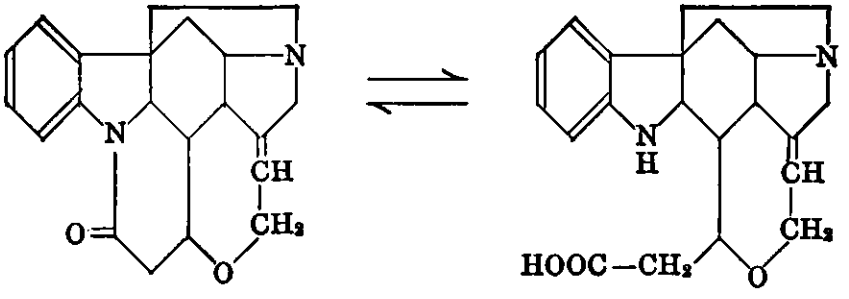
Strychnine

$$=NH \qquad HOH_2C— \qquad =N—CH_2—$$

Tetrahydrostrychnine Strychnidine strychnoline

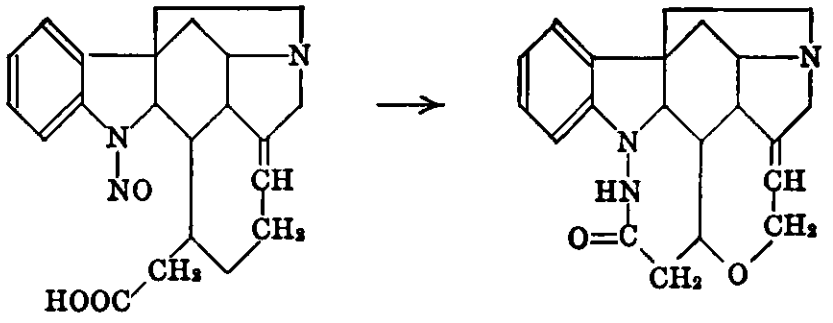
Because strychnine forms a benzylidene-derivative, an active methylene group must be adjacent to the amide group and must therefore be part of a heterocyclic ring. The second oxygen of strychnine cannot be detected with the usual reagents for carbonyl or hydroxyl groups. It is inert, and it is retained in strychnidine but eliminated in desoxystrychnine. It is now thought to form a cyclic ether linkage.[13]

When strychnine is treated with sodium ethoxide, it hydrates with the formation of strychninic acid, $C_{21}H_{24}O_3N_2$ (m.p.: 215°),[14] which on treatment with acids reforms strychnine. Because of the ability of that compound to form a nitrosamine and to undergo esterification, the change is thought to involve the nitrogen atom of the lactame group $N_{(a)}$ in the following reaction:



Strychnine Strychninic acid

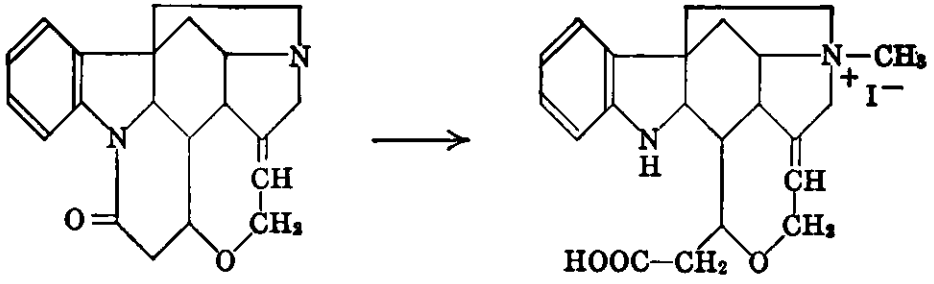
When strychnine is warmed with barium hydroxide instead of with sodium ethoxide, isostrychninic acid results, which is not able to reform strychnine but forms isostrychnine instead [15] (m.p.: 224°). This compound differs from strychnine by containing an hydroxyl group.[16] Oesterlin and Imoudsky [17] found also that strychninic acid passes easily into strychnine, but N-oxide-strychninic acid does not form strychnine-N-oxide by the action of mineral acids. N-nitroso-strychninic acid could be reduced, however, to the following compound:



These authors also synthesized amino-strychnine from an addition compound of N-oxide-strychninic acid and diazo-benzene-sulfonic acid.[17]

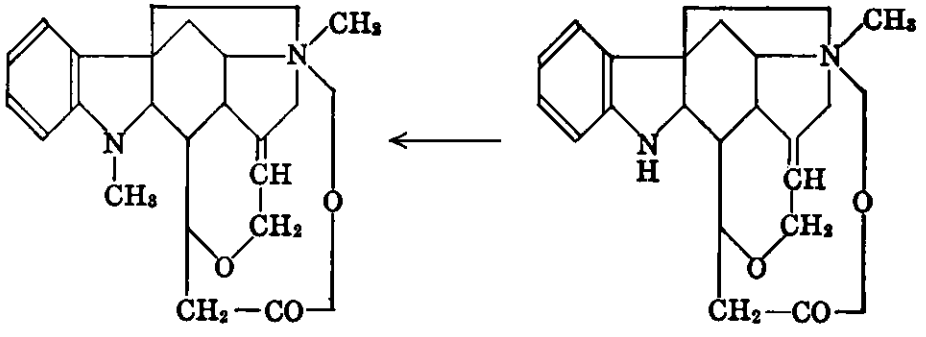
Isostrychnine-I and isostrychninic acid have been described by Leuchs and Schulte [18] and by Oesterlin and Imoudsky.[19]

Methylstrychnine, $C_{22}H_{26}O_3N_2 \cdot 2H_2O$, can be obtained from either strychnine methiodide or strychninic acid methiodide.[20] Dimethylstrychnine, $C_{23}H_{28}O_3N_2 \cdot 6H_2O$, has been obtained from both methylstrychnine methiodide or from strychninic acid.[21] Because of the reactions of this base, a N-methyl-tetrahydroquinoline nucleus had been suggested to be present in strychnine.[22]



Strychnine

Strychninic acid methiodide



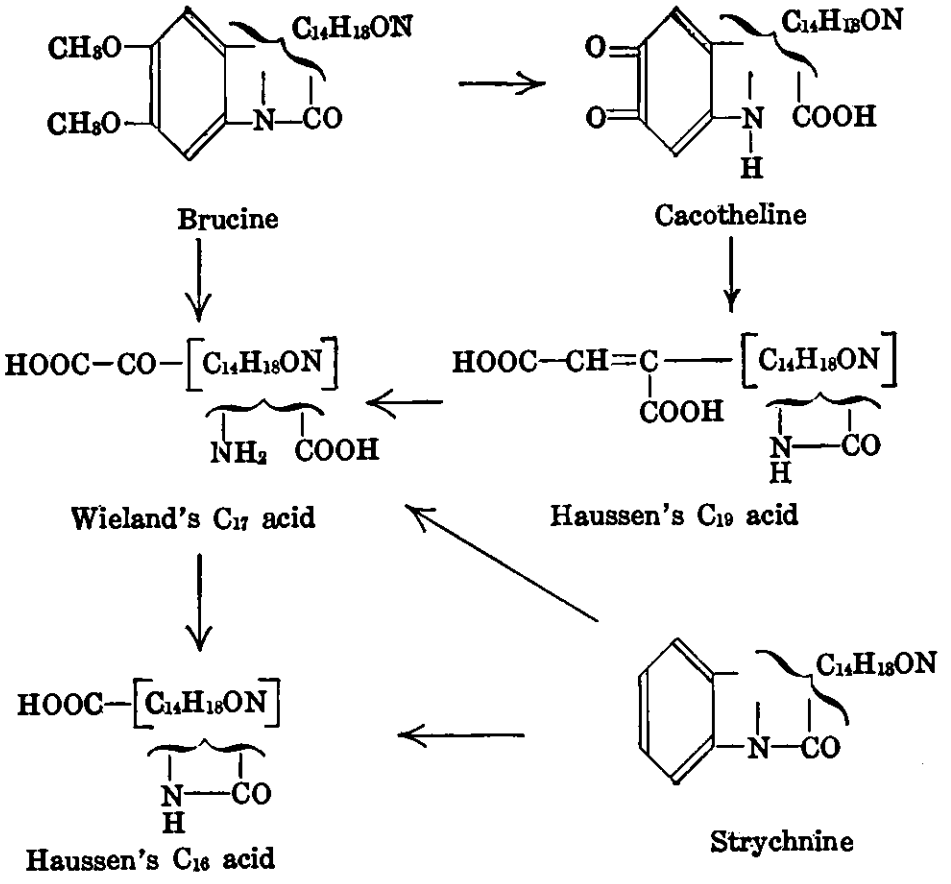
Dimethylstrychnine

Methylstrychnine

Oxidation Products of Strychnine and Brucine

The oxidation of strychnine and brucine was first investigated by Hanssen,[23] who also suggested the presence of a benzol-nucleus in both

compounds and the formulation of dimethoxy-strychnine for brucine, findings which were later confirmed and which can be summarized as follows:



Cacotheline has been extensively investigated.[24] It yields an oxime, can be reduced to a diaminophenol, and forms a methyl ester and a methosulfate.

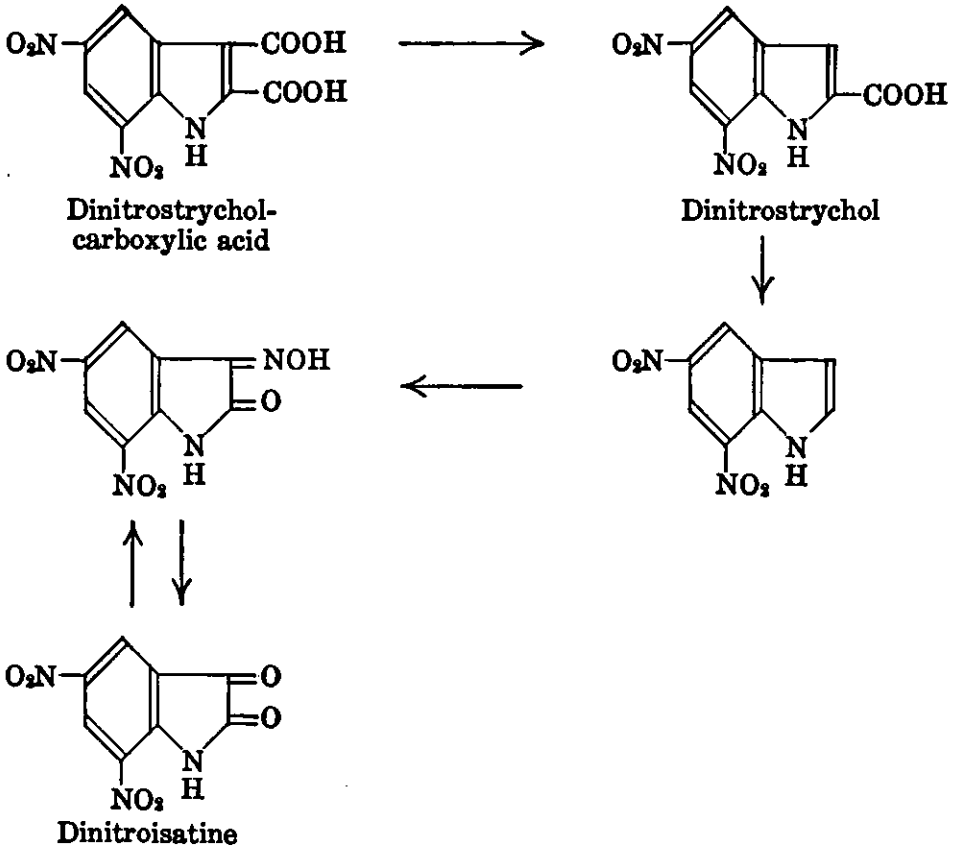
Hannsen's acid,[25] $C_{19}H_{22}O_6N_2 \cdot 2H_2O$, forms a methyl ester, and *d*-dimethyl ester. Different reduction products are obtained under different conditions, and the acid can be oxidized further as indicated.

Wieland's acid, $C_{17}H_{22}O_6N_2 \cdot 5H_2O$, has been obtained and investigated by several workers.[26] In the course of its catalytic hydrogenation, one mole of hydrogen is adsorbed and a lactame group is formed by loss of water, giving rise to the acid $C_{17}H_{22}O_5N_2 \cdot 3H_2O$. Oxidation yields the following acid:[27]

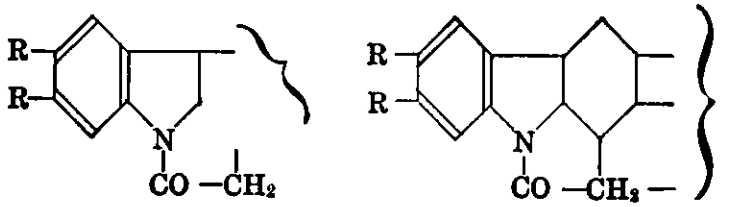
Hanssen's acid, $C_{16}H_{20}O_4N_2 \cdot 2$ or $4H_2O$

The degradation of strychnidine has been examined by Leuchs and coworkers,[28] who have also suggested a new nomenclature of the different derivatives, based upon two hypothetical bases, nucine and nucidine.

Dinitrostrycholcarboxylic acid, $C_{10}H_5O_8N_3$, obtained by Tafel,[29] can be reduced to the diamino-derivative, and it can also be decarboxylated to dinitrostrychol, $C_9H_5O_6N_3$, which must be a dinitro-indole-dicarboxylic acid, because it can be degraded further to picric acid.[30] Its formula has been definitely established by Menon and Robinson [31] and Hill and Robinson.[32] The following formulae present an outline of their work:



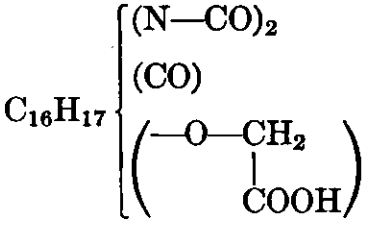
Based on the foregoing formulations and other information obtained by Spaeth and Bretschneider,[33] Lions *et al.*,[34] and others,[35] partial formulae for strychnine and brucine were suggested as indicated below:



R=H Strychnine
R=OCH_3 Brucine

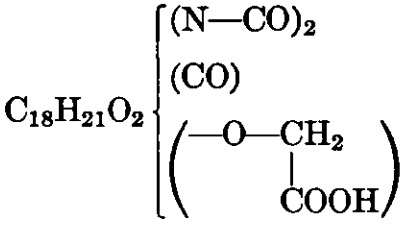
The following compounds have made it possible, in part, to elucidate the remaining part of the strychnine and brucine molecule.

Strychninonic acid, $C_{21}H_{20}O_6N_2 \cdot 2H_2O$, was obtained by the oxidation of strychnine with potassium permanganate.[36] Both nitrogens are bound in the form of lactame groups, and the compound shows no basic properties. It can be reduced to strychninolic acid, $C_{21}H_{22}O_6N_2$, which, in turn, can be hydrolyzed to strychninolone, $C_{19}H_{18}O_3N_2$, and glycollic acid.[37] Strychninolone occurs in three isomeric forms. A similar series of transformations can be undertaken, starting with brucine,[38] and several explanations have been offered,[39] but the following partial configuration has finally been adopted.[40]



Strychninonic acid

The degradation of brucinolone to curbine, $C_{18}H_{20}O_5N_2$, and the conversion of brucinonic acid to brucinolone have been described and discussed by Leuchs and his coworkers,[41] and the following system has been suggested for the parent alkaloids:



Brucinonic acid

The further oxidation of brucinonic acid has been investigated by Leuchs.[42] The oxidation of other products has been investigated with a view of determining the attachment of the $—CH_2—CH_2—$ chain, one end of which is attached to the nitrogen $N_{(b)}$.[43]

A shift of the double bond of strychnine, strychnidine, brucine, and other compounds, which can be accomplished in various ways, and gives rise to the neo-series of bases, has been described by several groups of workers.[44] Most recently strychnine and brucine have been converted into their neo-isomers by the action of Raney-nickel in boiling xylene.[45] Other developments which need to be mentioned briefly are an attempt to explain the formation of isostrychnine,[46] a proof of the relationship of brucine and strychnine,[47] the identification of beta-3-indolethylamine and 4-methyl-3-ethyl-pyridine among the degradation products of strychnine,[48] the investigation of pseudostrychnine and pseudobrucine,[49]

and the investigation of other degradation products of strychnine and brucine.[50]

Formulae for strychnine and brucine have been suggested by Perkin and Robinson,[51] Leuchs,[52] Blount and Robinson,[53] and Menon and Robinson.[54]

Evidence has recently been presented by Prelog and Szpilfogel to show that the ring E of strychnine, involving $N_{(b)}$ must be six-membered, because by the suitable degradation of strychninonic acid through an amino acid and an aldehyde, involving shortening of the chain, the ring E can be reformed readily, indicating that it must have consisted of at least six members prior to degradation.[55]

Other secondary alkaloids found together with strychnine and brucine are pseudostrychnine,[56] alpha- and beta-colubrine,[57] and vomicine.[58]

NATURAL SOURCES

Strychnine and brucine are usually made from *Strychnos nux vomica*, the seeds of which are official in the *National Formulary* (8th Edition). They are also known as poison nut, Quaker button, or dog button. The seeds of *Strychnos ignatii* Bergius, also of the family of Loganiaceae, have also been used occasionally, and they were formerly official in the *National Formulary* (5th Edition).

The *Strychnos nux vomica* tree grows wild in Indian forests, along the Malabar and Caromandel coast, in the hilly country of Travancore and Cochin, in Siam, Burma, and Ceylon, and in the northern parts of Australia. It is of moderate height, has a crooked trunk, numerous irregular branches, and a smooth, yellowish to grayish bark. The leaves, which are found on the younger branches, are opposite, ovate, entire, and have an acute apex. The flowers, which appear in the winter, are greenish white and grow in terminal corymbs. The fruit is an orange-yellow berry, about the same size as an orange, and contains up to five seeds in its starchy pulp.

The fruits are gathered when they appear to be ripe, and the pulp is separated from the seeds either by washing it off or allowing it to rot off. The seeds are then washed and are finally dried in the sun. They are packed in bags and exported from Bombay, Cocanada, Madras, Cochin, and Calcutta.

Nux vomica varies greatly depending upon its geographic origin.[59] It is described in the *National Formulary* (8th Edition) as follows: "The seed is orbicular, nearly flat, occasionally somewhat bent, from 10–30 mm in diameter, and from 3–6 mm in thickness. Externally it is grayish, or from pale brown to pale olive and is covered with appressed hair,

giving it a silky luster. The hilum is indicated by a circular scar at the center of the seed, and is connected with the micropyle by a radial raphe. The seed is very hard when dry. Beneath the thin and hairy seed coat is a large yellow to weak yellowish green endosperm, at one end of which is embedded a small embryo with two cordate 5- to 7-nerved cotyledons."

Nux vomica is also official in the *British Pharmacopœia*. *Nux vomica* Extract, Fluid Extract, and Tincture are recognized in both countries In addition, powdered *Nux vomica* is recognized in the *British Pharmacopœia*, and *Nux vomica* Alkaloid Solution is recognized in the *National Formulary*, which also recognizes Brucine Sulfate.

Strychnine and the following preparations are official in the *National Formulary* (8th Edition): Aloin, Strychnine and Belladonna Pills; Aloin, Strychnine, Belladonna and Cascara Pills; Aloin, Strychnine and Ipecac Pills; Compound hypophosphites syrup; Strychnine nitrate and strychnine nitrate tablets and strychnine phosphate. The *U. S. Pharmacopœia* recognizes strychnine sulfate and strychnine sulfate tablets. The *British Pharmacopœia* recognizes strychnine hydrochloride and solution of strychnine hydrochloride.

MANUFACTURING METHODS

Preparation of the crude drug prior to extraction of the alkaloids, and the extraction of the alkaloids itself, can be undertaken in a number of different ways, all of which give equally good results. Those that are most feasible on a technical scale will be described here.

The following section can be subdivided into three headings: (1) Treatment of the Drug prior to Extraction; (2) Extraction and Separation of the Alkaloids; (3) Purification of the Bases and the Manufacture of Their Different Salts.

Treatment of the Drug Prior to Extraction

Generally speaking there are two possible ways of preparing the drug for extraction: Softening of the *Nux vomica* prior to or after grinding. Because of the vast technical improvements in recent years of the grinding and milling machinery available, softening of the drug prior to milling does not seem to offer any special advantages at this time. Treatment of the *Nux vomica* with a hot solution (80–90°) of slaked lime for 24 hr seemed advantageous because of the extremely hard consistency of the nut. This treatment softened the nut and prolonged considerably the life of the machinery involved. However, it does not soften the interior of the nut to the same extent as a similar treatment of the crushed nut would, with the result that extraction of the alka-

loids is somewhat more difficult and prolonged. In addition, milling of the damp material is not quite as effective as milling of the dry nuts. Material which ordinarily would be removed as a more or less fine powder now sticks inside the mill in the form of a slimy paste. The only alternative, drying of the pretreated nut in a rotary steam-heated dryer, involves a considerable expense of time and labor.

The crude drug is first crushed in a strong mill to relatively small pieces of about the size of peas. This milled drug is then transferred to an iron kettle, equipped with a jacket and a slow but efficient stirrer, containing a number of horizontal arms at intervals all along the shaft. A similar kettle equipped with a head and a tight manhole, built to withstand some 30–40 lb of pressure, is even preferable. The material is then covered with enough of a solution of slaked lime, so that the resulting mixture is a fairly thin paste. Stirring is continued and heat is applied until the mixture reaches 80–90°. If an open kettle is used, heating and stirring for about 24 hr are recommended; in a closed kettle about half that time is usually sufficient. If the paste becomes too thick during this heating, more milk of lime can be added. At the end of the time allotted, the material is immediately ready for extraction. Any excess water can easily be removed and should be used to slaken the lime for the next batch.

It has also been recommended simply to treat the whole nut with live steam to soften it down, but this method has not been too successful.

Extraction and Separation of the Alkaloids

Extraction of the alkaloids is preferably undertaken in the same type of a vessel in which the softening operation described before is performed. The extractor should be equipped with a stirrer and a jacket for heating with steam. The percolator described in Chapter I can be used, provided that provisions are made to introduce the solvent at different points within the extractor, by use of pipes inserted either through the jacket or inside the extractor along the sides or in the center. Circulation of the liquid used for extraction for several hours will help greatly to remove all the alkaloids. Extractors of the basket type can also be used, and here again the solvent should be circulated for some time.

A variety of solvents can be used for the extraction.

a. Water-miscible Solvents. Included in this category are alcohol and dilute sulfuric acid. In the case of alcohol, methanol, ethanol, or isopropanol would be the logical choice. The hot alcohol is circulated for some time, and three or four extractions will usually be necessary

to remove all the alkaloids from the crude drug. The combined extracts are evaporated under ordinary pressure. The residue from this distillation is dissolved in dilute sulfuric acid, and this solution is then ready for further processing. Extraction with dilute (3%) sulfuric acid cannot be recommended, mainly because of its extremely corrosive action upon all parts of the equipment used. Theoretically, the drug should be extracted with dilute sulfuric acid at 80–90° three or four times, and this acid aqueous extract would be ready directly for further processing. Although the liquor could be used over again several times, it would have to be extracted with a solvent from time to time to remove some of the alkaloids, which are somewhat soluble in water.

b. Water-immiscible Solvents. Included here are naphtha, chloroform, toluol, and benzol, but the first and the last two mentioned are the only ones of commercial importance. Because of the health hazards involved in using large amounts of hot benzol, that solvent cannot be recommended and toluol is preferred not only because of that reason but also because of its higher boiling point. However, naphtha, or kerosene, or any similar hydrocarbon mixture is most desirable. The reasons for its preference are its relatively high boiling point, its high flash point, its nontoxicity, the easy way in which it can be recovered from the exhausted drug, and its low price.

The drug is thoroughly mixed with the solvent for several hours, with intensive warming, after which time the solvent is drawn off and transferred to a mixer, where it is extracted with dilute sulfuric acid (3–5%) until all the alkaloids have been removed. The solvent is then returned to the crude drug for further extractions, of which three or six are necessary as a rule. A simple way to test for completeness of extraction is to draw a small sample of naphtha or toluol, extract it with dilute sulfuric acid, and add ammonia to that aqueous sample. The presence of only an opalescence but of no precipitate indicates completeness of extraction. If this is the case, the last traces of solvent are removed from the drug, after the main portion has been drawn off, by means of live steam, which is then passed through an efficient condenser and the distillate collected in a tank equipped with a bottom outlet, to permit the water to be drawn off.

When all the alkaloids have been removed from the organic solvent by means of dilute sulfuric acid, the total aqueous acid solutions are combined, and the alkaloids are precipitated slowly and under constant stirring with a solution of sodium carbonate, sodium hydroxide or even ammonia. Stirring is continued for a while, and the precipitated alkaloids are then allowed to settle overnight. The clear supernatent liquid is then syphoned or pumped off. Some of it can be used over

again in treating another batch of ground *Nux vomica*. The rest, which still contains some alkaloid, is slightly acidified and concentrated in an open evaporator. The cooled liquor is made strongly alkaline and is then extracted with toluol or another solvent such as naphtha. If the aqueous solution is void of alkaloids, it can be discarded; the solvent solution is returned to the process at an appropriate point.

The suspension of crude alkaloids in the alkaline motherliquor is transferred to a filter or centrifuge and drained as much as possible. The filtrate is added to the aqueous liquor and processed as described before. The crude alkaloids are transferred to a still or kettle, which is equipped with a stirrer and connected to a pressure filter as described in Chapter I. To this material is now added sufficient alcohol, so that the material will finally be dissolved in approximately 1½ parts of about 50% alcohol. If the crude material is considered to contain an average of about 30% of moisture, then for each 100 lb of the crude damp alkaloids approximately 25 lb of water and about 60–70 lb of alcohol will have to be added. The mixture is then refluxed until everything is in solution, with the exception of small amounts of inorganic material occasionally present. At that point a small amount of charcoal and filteraid is added, and the hot mixture is filtered through the pressure filter. The filtercake is washed with a small amount of 50% alcohol and discarded. The washing is combined with the original filtrate, and the strychnine is allowed to crystallize. The brucine will mostly remain in the motherliquors. Crystallization should proceed at room temperature overnight.

Instead of denatured alcohol, methanol or isopropanol can be used, but the concentration must be somewhat changed, especially in the case of the latter.

When the crystallization of the semicrude strychnine is complete, the material is filtered or preferably centrifuged off. If a stoneware filter is used, the material should be pressed down hard to expel the last traces of motherliquor containing the brucine. In the centrifuge the material is simply centrifuged to near dryness. In neither case is the crude strychnine washed.

From the motherliquors the brucine can be isolated in a number of different ways. After acidification with sulfuric acid, all the alcohol and some of the water can be removed by distillation in a glass-lined still, and after crystallization the brucine sulfate can be filtered off. A more complete separation can be achieved by carefully neutralizing the motherliquor with oxalic acid, and permitting the oxalates of the total alkaloids present to crystallize. After filtering and drying, the oxalates are treated with absolute ethanol or isopropanol, whereby the

strychnine oxalate passes into solution. The remaining brucine oxalate is filtered off, suspended in a small amount of water, and the brucine is precipitated by the addition of alkali. By still another method the brucine oxalate is treated with charcoal in a hot aqueous solution, and the clear filtrate is mixed with magnesium oxide and evaporated to dryness. From the residue the brucine can be extracted by means of acetone or other solvents.

The separation of strychnine and brucine can also be effected by crystallization of the sulfates. The solvent is extracted with hot, dilute sulfuric acid (5%) in such a manner that too much an excess of the acid is avoided. The hot solution, which is acid to congo test paper, is then treated with charcoal, filtered, and allowed to crystallize above 25°. Under these conditions, when the solution is not too concentrated, or when the solution does not cool off to below 25°, only brucine sulfate will crystallize. The strychnine sulfate will stay in solution and can be recovered from the filtrate in the usual manner, either by concentration and crystallization of the sulfate or by precipitation of the base. Further purifications of both the brucine and strychnine are usually necessary.

Purification of the Bases and Preparation of Their Salts

Prior to preparation of pure strychnine base and of its salts, the crude strychnine alkaloid described before should be crystallized once more.

The crude strychnine is dissolved in a sufficient amount of dilute sulfuric acid (10%) to effect solution, but the resulting solution should only be slightly acid to congo test paper. The solution is then treated with a small amount of charcoal and filteraid, and after heating to about 80° for one hour it is filtered through a pressure filter, and the filtercake is washed with a small amount of hot water. Filtrate and washings are combined and allowed to crystallize. They are then filtered or centrifuged off, and the strychnine sulfate is washed with a small amount of ice water. Motherliquor and washings are combined, and the residual strychnine, containing some brucine, is precipitated with alkali, filtered off, dried, and stored until a considerable quantity of that material has been collected. The motherliquor, as all other aqueous motherliquors, should be freed of all alkaloids by extraction with a solvent, if necessary, before discarding. The above-mentioned mixture of strychnine and brucine can be resolved into its components by crystallization from either 25, 50, or 80% alcohol. In either of these solvents the brucine is more soluble and will remain in solution. The strychnine thus obtained will need further purification through the sulfate to remove the last traces of brucine.

If the semicrude strychnine sulfate obtained above still contains more than slight traces of brucine, it will have to be recrystallized again, which is easily accomplished by dissolving it in five parts of water at 80°, filtering with charcoal and filteraid, and allowing the strychnine sulfate to crystallize. This material is usually pure enough to prepare pure strychnine alkaloid, sulfate, or bisulfate. The other salts are prepared from the pure alkaloid.

The Manufacture of Pure Precipitated Strychnine. The strychnine sulfate mentioned before is dissolved in approximately ten times its weight of hot water (80°), and the solution is made exactly neutral to litmus test paper by the careful addition of a solution of sodium carbonate. The hot solution is then treated with a small amount of charcoal and filteraid, filtered through the pressure filter, and the base is precipitated by the addition of an excess of sodium carbonate and simultaneous cooling. After cooling is complete, the base is filtered off, washed with ice water, and dried at a reasonable temperature.

The Manufacture of Pure Crystalline Strychnine. Semicrude strychnine sulfate is dissolved in about eight times its weight of about 30% ethanol, but methanol or isopropanol of about the same dilution can also be used; in the case of isopropanol the solution should be about 35–40%. The mixture is heated to dissolve all strychnine sulfate (reflux condenser should be used), sufficient concentrated aqueous ammonia is added to render the solution slightly alkaline to litmus test paper, and after filtration with charcoal and filteraid the solution is made strongly alkaline with ammonia and is allowed to cool slowly. Water may be added gradually to the solution if especially well-formed crystals are desired.

The Manufacture of Neutral Strychnine Sulfate. The semicrude strychnine sulfate is dissolved in about six times its weight of hot water, and the solution is adjusted to a very slight acid or practically neutral reaction by adding concentrated ammonia. After filtration with charcoal and filteraid, and subsequent cooling, the crystalline material is filtered off, washed with a small amount of ice water, and dried. The motherliquors and washings are combined, and the strychnine is precipitated therefrom.

The Manufacture of Strychnine Bisulfate. Ten parts of semicrude strychnine sulfate are dissolved in sixty parts of warm water, the solution is rendered slightly alkaline by the addition of concentrated ammonia, and then strongly acid again by the addition of 1.4 parts of concentrated sulfuric acid. After treatment with a small amount of charcoal and filteraid and filtration, the salt is allowed to crystallize with cooling and stirring. It is filtered, washed with a small amount of ice water,

and dried. The motherliquors and washings are combined as before, and some strychnine is recovered by precipitation with alkali.

The Manufacture of Strychnine Nitrate. In the preparation of this salt, an excess of nitric acid must be avoided. One part of the base is suspended in about five parts of water, and approximately 95% of the theoretical amount of concentrated nitric acid (as calculated) is added slowly under stirring. The solution is then made acid to litmus by the addition of an organic acid, such as acetic or tartaric. After heating with charcoal and filteraid and filtration, the strychnine nitrate is allowed to crystallize not below 25–30° to avoid possible crystallization of the organic acid salt of strychnine.

The Manufacture of Strychnine Arsenate. One part of strychnine is suspended in a small amount of water and brought into solution by the cautious addition of tartaric or formic acid. The final solution, which should be small in volume, should be slightly acid to litmus test paper. To keep the volume low, the solution can be warmed. In a separate vessel a solution of 0.35 part of arsenic pentoxide in the theoretical amount of potassium hydroxide is prepared. After filtering the two solutions separately with charcoal and filteraid, they are combined and, if necessary, adjusted to slight acidity with an organic acid. Upon cooling, strychnine arsenate will crystallize with one half mole of water of crystallization. After filtration it can be washed with a small amount of ice water first and then with some cold alcohol, whence the anhydrous compound is obtained. The hydrated form is efflorescent in dry air. One part is soluble in 14–20 parts of water, but the compound is only slightly soluble in alcohol. It has also been reported to crystallize with two moles of water of crystallization.

The Manufacture of Strychnine Glycerophosphate. A solution of strychnine in a dilute organic acid is prepared and filtered as described before. To it is then added a slight excess over the calculated amount of glycerophosphoric acid, and the strychnine salt is allowed to crystallize. This compound crystallizes with six moles of water of crystallization, and it is only slightly soluble in water or alcohol. Its description can be found in the *National Formulary* (4th Edition) or in the *U. S. Dispensatory* (21st Edition), page 1493.

The Manufacture of Strychnine Hypophosphite. This salt of strychnine can be prepared by dissolving the exact amount of strychnine in a strong aqueous solution of hypophosphorous acid, and concentrating to dryness after filtering with charcoal and filteraid. The compound containing two moles of water of crystallization can be obtained by crystallization from 60–70% alcohol. It should be air-dried.

Other Salts of Strychnine. The salts of that base with phosphoric, valeric (*National Formulary*, 4th Edition), gluconic, or other acids are prepared similar to the ones mentioned previously.

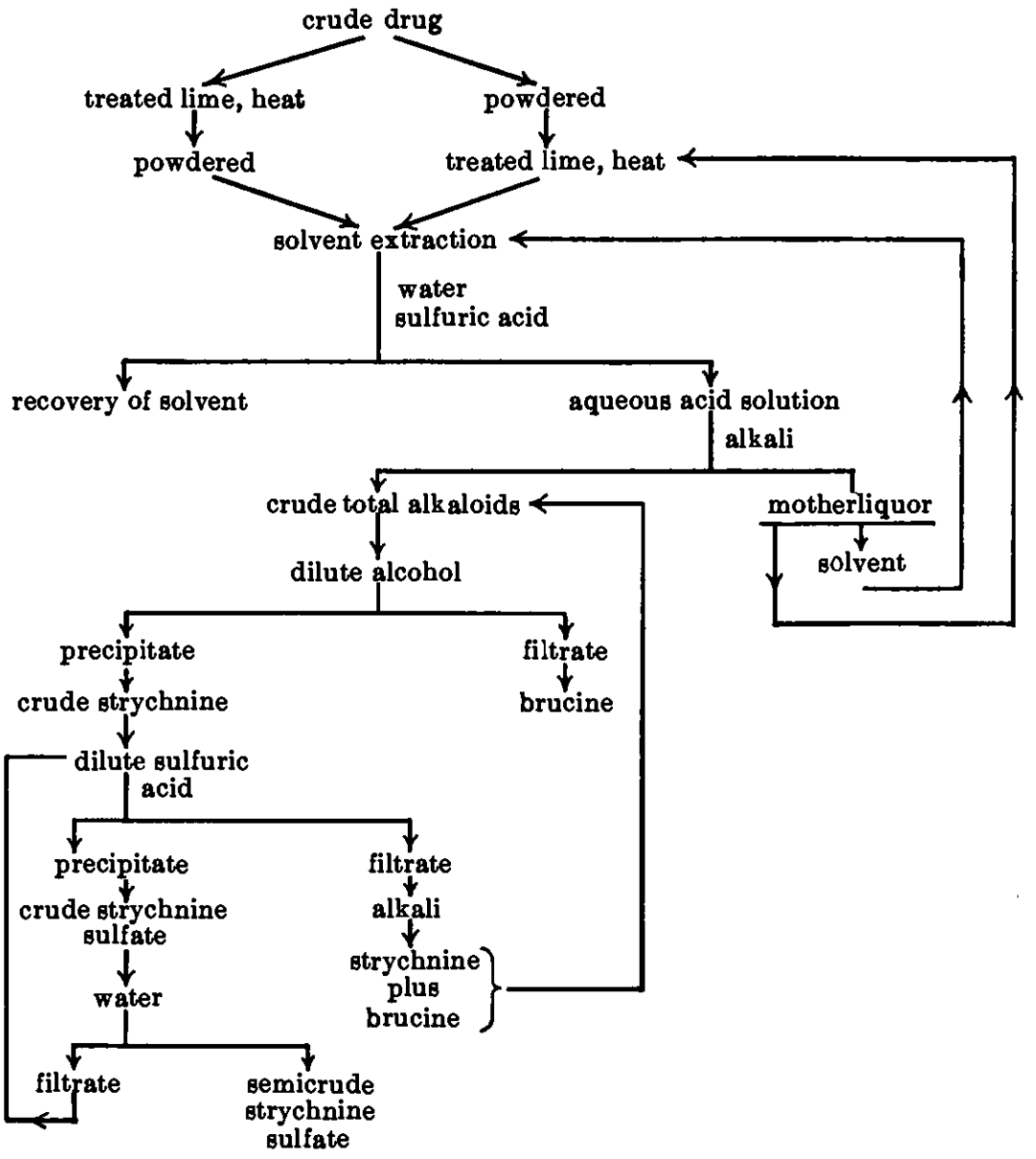
The Manufacture of Pure Brucine. The purification of the crude brucine obtained from the motherliquors of the manufacture of strychnine is best purified by crystallization of its neutral oxalate from a hot (80°) aqueous solution after filtration with charcoal and filteraid. Washing the crystalline precipitate on the filter with a small amount of cold alcohol will serve to separate traces of strychnine. Brucine can then be precipitated from a warm solution of its salt by means of an excess of ammonia.

Brucine can also be purified by triturating the crude base with small amounts of cold acetone, ethanol, or isopropyl alcohol, either of which will dissolve the brucine and leave the strychnine behind. After filtration, the filtrate is warmed and diluted with warm water until the brucine begins to crystallize. Because brucine is somewhat more soluble than strychnine, all motherliquors from its preparation or from the preparation of its salts should be treated with alkali and extracted with a water-immiscible solvent to remove the last traces of the alkaloid.

Other means of separating traces of strychnine from brucine take advantage of the slight solubility of the following salts of the former base in aqueous solution: the chromate from almost neutral solution or the potassium ferrocyanide compound from dilute hydrochloric acid. The brucine can be precipitated from the filtrate as usual. It can finally be crystallized from dilute alcohol. The base has also been obtained by crystallization of brucine hydrochloride from alcoholic ammonia.

The Manufacture of Brucine Sulfate. This salt can easily be obtained by adding sulfuric acid to a warm (70–80°) aqueous suspension of the purified alkaloid until the resulting solution is slightly acid to congo test paper. After filtration with charcoal and filteraid, a small amount of sodium bisulfite is added to the filtrate to avoid discoloration of the crystallizing salt. If necessary, brucine sulfate can be recrystallized from eight to ten parts of boiling water. This salt is official in the *National Formulary* (8th Edition), where its description can be found.

The Manufacture of Other Salts of Brucine. Other salts of brucine are prepared similarly to the corresponding salts of strychnine, but they are usually more soluble than the latter. Besides the sulfate, the hydrochloride and nitrate are usually prepared. All of these salts are soluble in water, but they can be crystallized from a very concentrated aqueous or alcoholic solution by the addition of acetone.



PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

Strychnine is a highly toxic alkaloid, and when it is administered in fairly high doses, death may result because of respiratory failure. Strychnine stimulates the whole nervous system, but especially the respiratory and vasomotor centers, and the spinal cord and possibly parts of the brain. The latter is evidenced by occasional wakefulness; stimulation of the spinal cord motor and sensory ganglia is evidenced by convulsions.[60] The paralyzing action on the motor nerves is similar to that of curare. The alkaloid raises the blood pressure and slows the heart. It is also said to act as a tonic by increasing the peristaltic activity.[61]

Strychnine has been used in certain cases of shock, collapse, or poisoning as either a circulatory or respiratory stimulant, but its value has been questioned.[62] The main use of strychnine is nonmedicinal, that of a vermin killer.

Brucine resembles strychnine in its action, but it is considerably less toxic. It paralyzes the peripheral motor nerve endings, but does not produce the convulsive reflexes characteristic of strychnine,[63] except in very large doses.[64] Brucine has an even more curare-like action than strychnine.

ANALYTICAL METHODS

Both strychnine and brucine dissolve without color in concentrated sulfuric acid. The former gives a yellow solution in concentrated nitric acid (brucine gives a red to orange solution), whose residue after evaporation turns purple with alcoholic potassium hydroxide. A solution of strychnine in concentrated sulfuric acid, when treated with potassium permanganate, potassium dichromate, or manganese dioxide, turns blue, violet, red, and then yellow. Organic matter and brucine often interfere with this reaction, and these impurities should be removed first if possible.[65] Brucine alone does not give the play of colors when oxidized with chromic acid.

Strychnine also gives the following reactions: a purple to orange color with ammonium vanadate dissolved in sulfuric acid dihydrate; a purple color with sulfuric acid and potassium ferricyanide; and yellow colors with sulfuric acid and sodium nitrite, sulfuric acid and potassium chlorate, and alcoholic potassium hydroxide. For its microchemical identification, crystalline precipitates have been obtained with different mono-, di- and trinitro benzoic acids, most of which show strong double refraction. Other microchemical methods including the differentiation of brucine and strychnine have been described by Martini.[66]

Brucine gives the following reactions in addition to the ones mentioned before: both Froehde's and Erdmann's reagents give a yellow or orange solution. Precipitates are obtained with iodine-potassium iodide, Mayer's reagent (1:30,000) aurichloride, potassium bismuth iodide, phosphomolybdic acid, tannic acid, and platinic chloride. The following precipitates can be used for its microchemical identification: a crystalline precipitate with meta-dinitrobenzoic acid and rosettes of prisms obtained with trinitrobenzoic acid.

The following characteristic microchemical precipitates have been described by Stephenson:[67] For brucine: with potassium hydroxide, needles; with platinic chloride, rods; with chromic acid, rods and rosettes. For strychnine: with potassium hydroxide, rods with V-shaped ends

(1:20,000); with sodium phosphate, small rods (1:1000); with ammonium thiocyanate, rods; with picric acid, large green rosettes; and variously formed precipitates with aurichloride, platinichloride and mercuric chloride.

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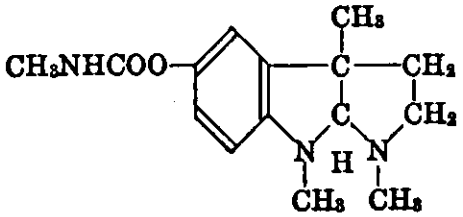
CHAPTER XV

PHYSOSTIGMINE AND RELATED ALKALOIDS

CHEMISTRY

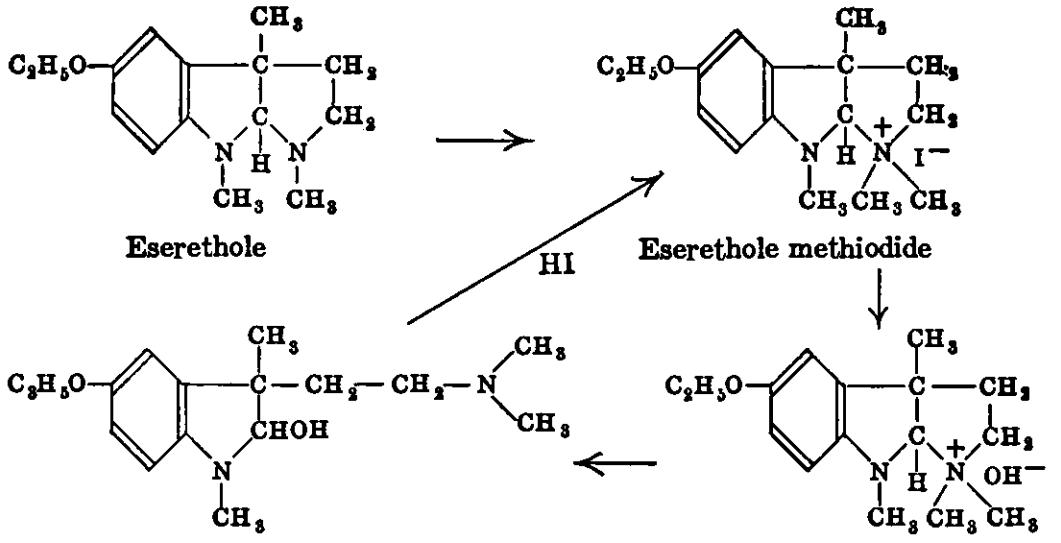
The seeds of *Physostigma venenosum* contain, as their main alkaloid, physostigmine, also known as eserine:



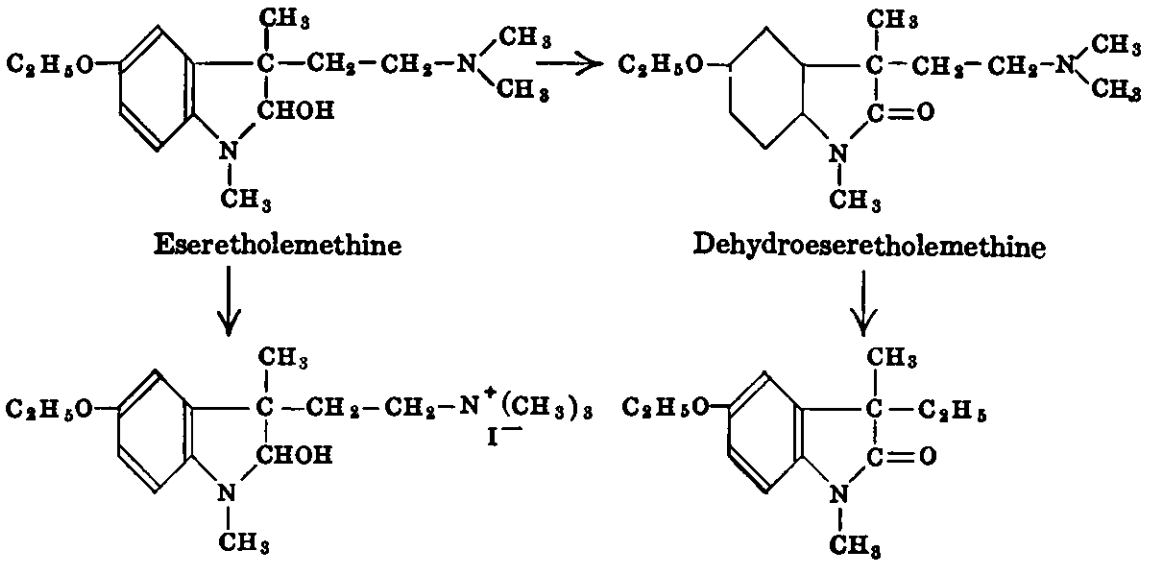
The secondary alkaloids, which accompany physostigmine, occur in small amounts and are of little importance. They are geneserine, eseramine, physovenine, and isophysostigmine.

The first product of the gentle hydrolysis of physostigmine is eseroline, which is obtained together with methylamine and carbon dioxide if the hydrolysis is performed with alkali, but if sodium ethoxide is used, methylurethane is obtained together with eseroline. Methylcarbimide is one of the products obtained when physostigmine is heated to its melting point. On the other hand physostigmine can be obtained from eseroline and methylcarbimide in the presence of sodium. These reactions give proof without any doubt of the presence of a urethane group in physostigmine.[1] Eseroline, like its parent substance, is a monoacidic tertiary base and contains two methylimino groups.[2]

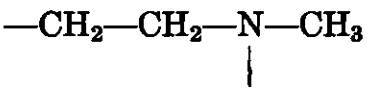
Eseroline contains a phenolic hydroxyl group which remains unchanged when eseroline methiodide is heated in an atmosphere of carbon dioxide to yield physostigmol, $C_{10}H_{11}ON$ (m.p.: 103°), which also contains a methylimino group.[3] Zinc and hydrochloric acid reduce eserethole to a secondary base, dihydroeserethole,[4] probably by the scission of a heterocyclic ring.[5] Eserethole methiodide on treatment with alkali yields eseretholemethine (m.p.: 89°), a reaction which can be reversed with hydriodic acid. Eseretholemethine, on treatment with methyliodide followed by Hofmann degradation, furnished trimethylamine and etheseroline, $C_{12}H_{12}N—OEt$ (m.p.: 48°). Polonovsky explained this series of reactions as follows:[6]



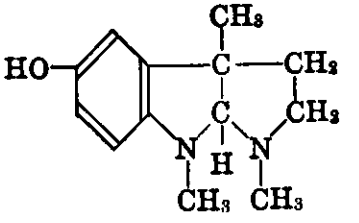
During the reduction of eserethole the pyrrolidine ring opens, forming dihydroeserethole. A phenomenon similar to the formation of eserethole methiodide from eseretholemethine had previously been observed with some alkylene indolines.[7] The change from eseretholemethine to methyleseretholinium hydroxide is a tautomeric one, taking place without the loss of water.[8] Proof of the indolinol character of eserethole-methine may be had from the formation of a diquaternary picrate with the simultaneous loss of the hydroxyl group. Oxidation of the former with potassium ferricyanide yields dehydroeseretholemethine, which on exhaustive methylation yields trimethylamine and an unsaturated product which can be hydrogenated to 5-ethoxy-1,3-dimethyl-3-ethyl-2-indolinone (m.p.: 68°).



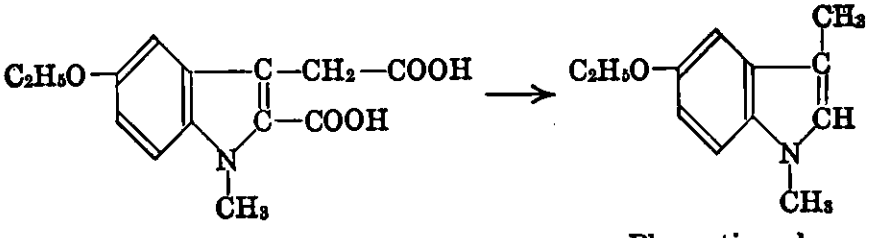
To obtain eseroline from physostigmol, figuratively speaking, the residue C_3H_7N must be added. In this residue the nitrogen must be basic and tertiary, and one N-methyl group must be present. One of the two possible formulae for that residue is, therefore, the following:



which implies the formation of a pyrroline ring. A formula for eseroline was proposed by Robinson [9] and confirmed by Barger and Stedman.[10]



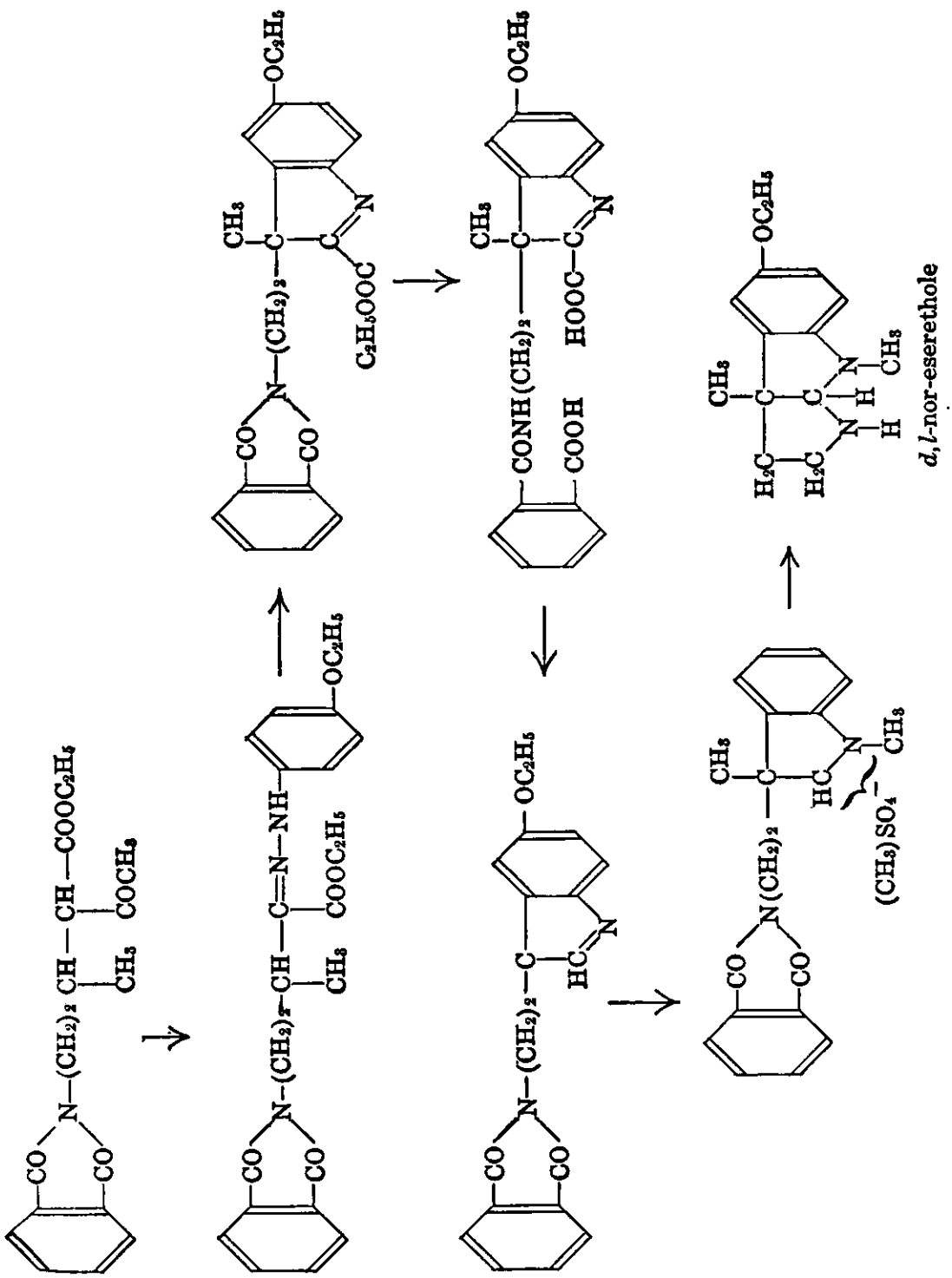
Physostigmol ethyl ether, which is formed by heating eserethole methiodide under vacuum, has been synthesized by Stedman.[11] Para-ethoxyphenylmethylhydrazine was condensed with alpha-ketoglutaric acid to form 5-ethoxy-2-carboxy-1-methylindole-3-acetic acid, which, on decarboxylation, gave 5-ethoxy-1,3-dimethylindole, identical with physostigmol ethyl ether.



Physostigmol ethyl ether

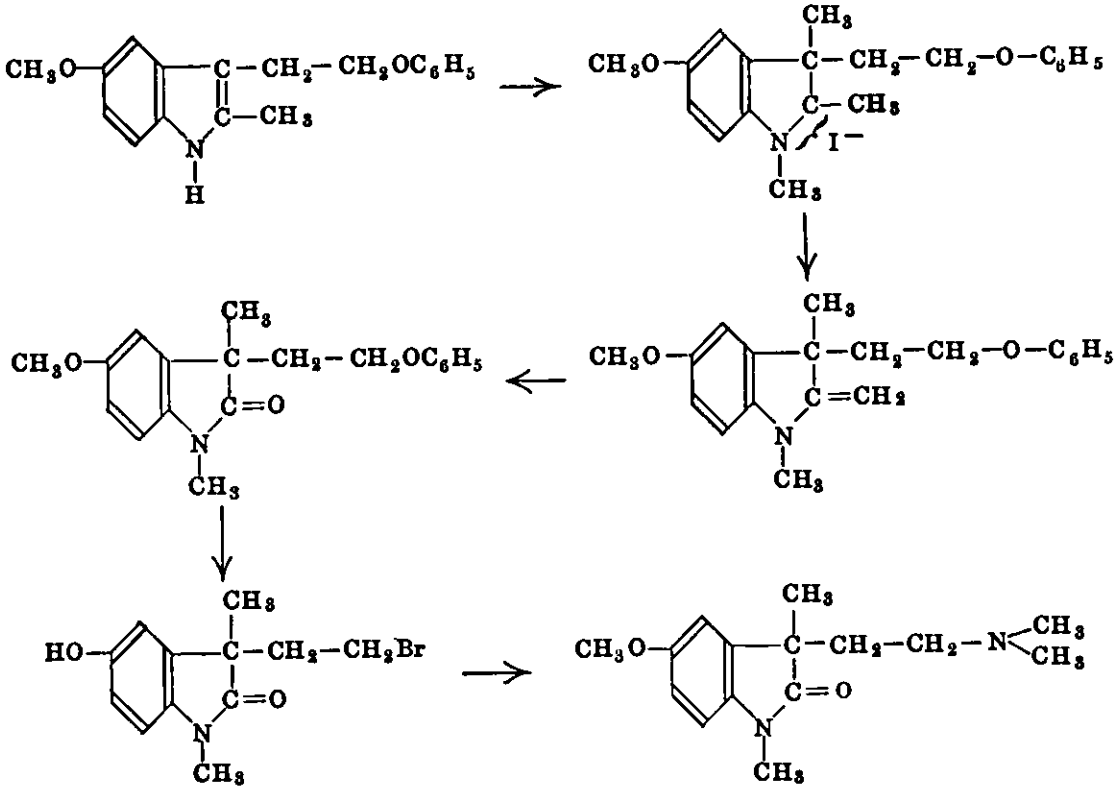
The structure of physostigmine has been confirmed by a number of total or partial syntheses.

The preparation of *d,l*-nor-eserethole has been achieved by Robinson and Suginome.[12] Phthalo-gamma-bromo-butylimide was condensed with sodio-ethylacetoacetate to form ethyl-delta-phthalimido-alpha-acetyl-beta-methyl-valerate, which served as the starting point of their synthesis, described in the following diagram:


d,l-nor-eserethole

King, Liguori, and Robinson [13] obtained the intermediate 5-ethoxy-3-methyl-3-(beta-phthalimidoethyl)-indolenine directly from gamma-phthalimido-alpha-methyl-butaldehyde and para-ethoxy-phenylhydrazine. The methosulfate of that base on treatment with hydrazine in alcohol split off phthalylhydrazide, and ring closure was effected with hydrochloric acid (last three formulae in preceding diagram).

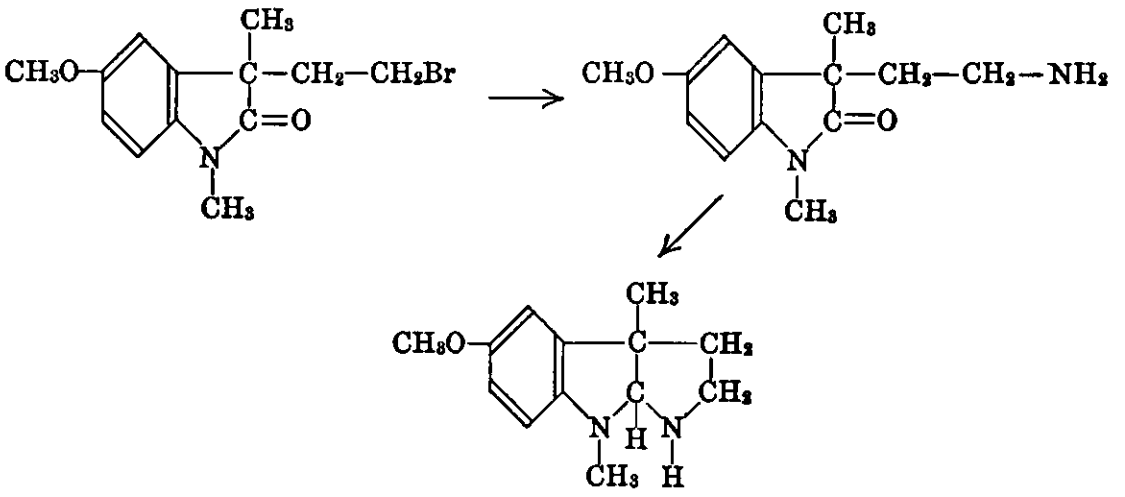
Dehydroesermetholemethine, the methoxy-derivative corresponding to dehydroseretholemethine, was synthesized by King and Robinson,[14] starting from the para-methoxyl-phenylhydrazone of gamma-phenoxy-propylacetone:



Dehydroesermetholemethine

The racemic base thus obtained was resolved into its optical antipodes, of which the methopicrates were prepared. The methopicrate of laevo-dehydroesermetholemethine was identical with the corresponding compound obtained from natural physostigmine.

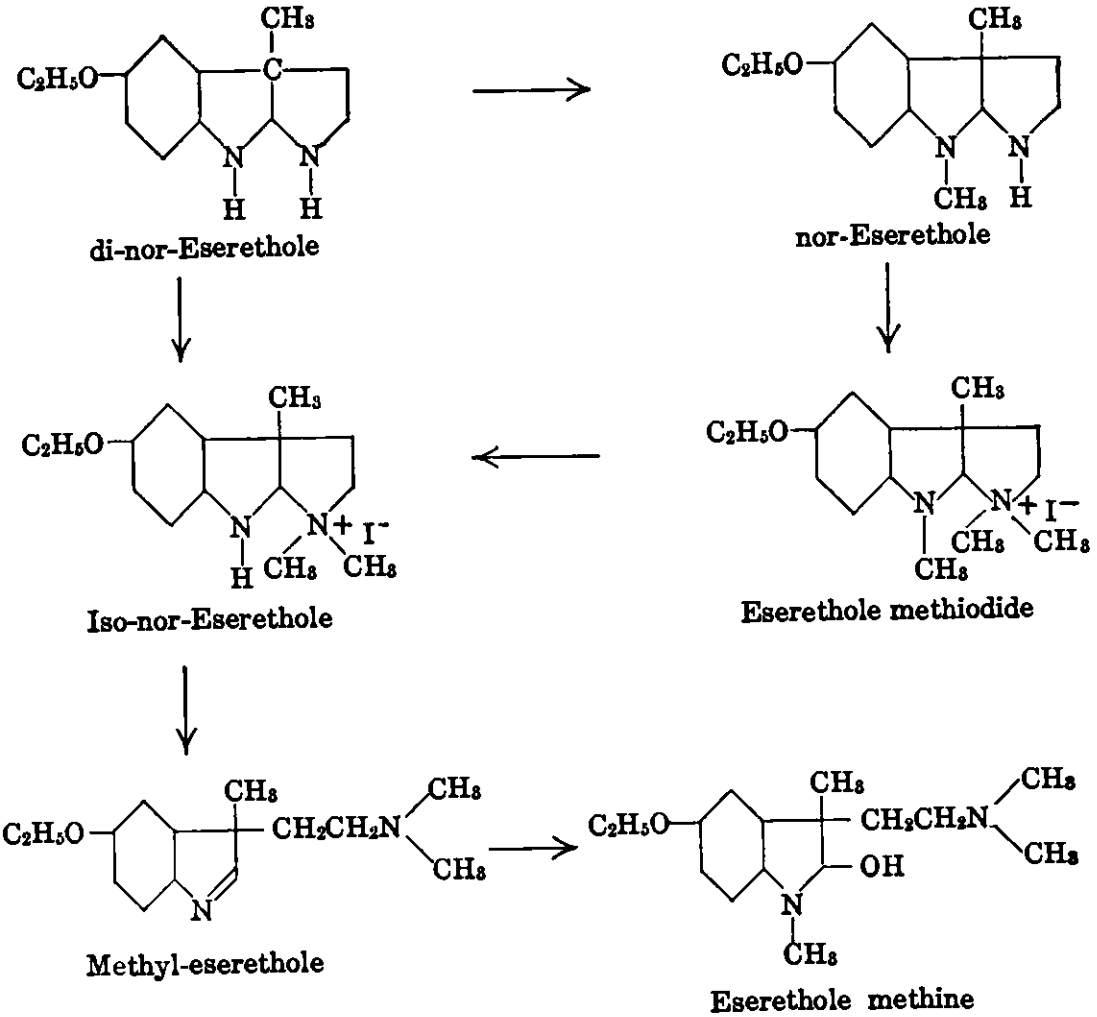
One of the intermediates in the foregoing synthesis, 5-methoxy-1,3-dimethyl-3-beta-bromoethyl-2-indolinone, was used by King *et al.*[15] to synthesize *d,l*-nor-esermethole:



d,l-Nor-esermethole

Methylation of this base yields *d,l*-esermethole, the methopicrate of which occurs in two forms, melting at 184° and 190° respectively. A mixture of these two compounds has the lower of the two melting points. The corresponding laevo-compound prepared from natural physostigmine melts at 194°, but mixed melting points with either of the synthetic compounds show the lower melting point of the synthetic compound.[16]

Racemic di-nor-eserethole was prepared by Hoshino and Kobayashi [17] from 5-ethoxyindolyl-beta-ethylamine by reaction with ethyl magnesium iodide and then with methyl iodide. As indicated below, a number of different reaction products could be obtained by methylation of di-nor-eserethole.[18] One of these products, methyleserethole, the structure of which was confirmed by synthesis,[19] proved to be different from the *d,l*-eserethole-b prepared by King *et al.*[20]

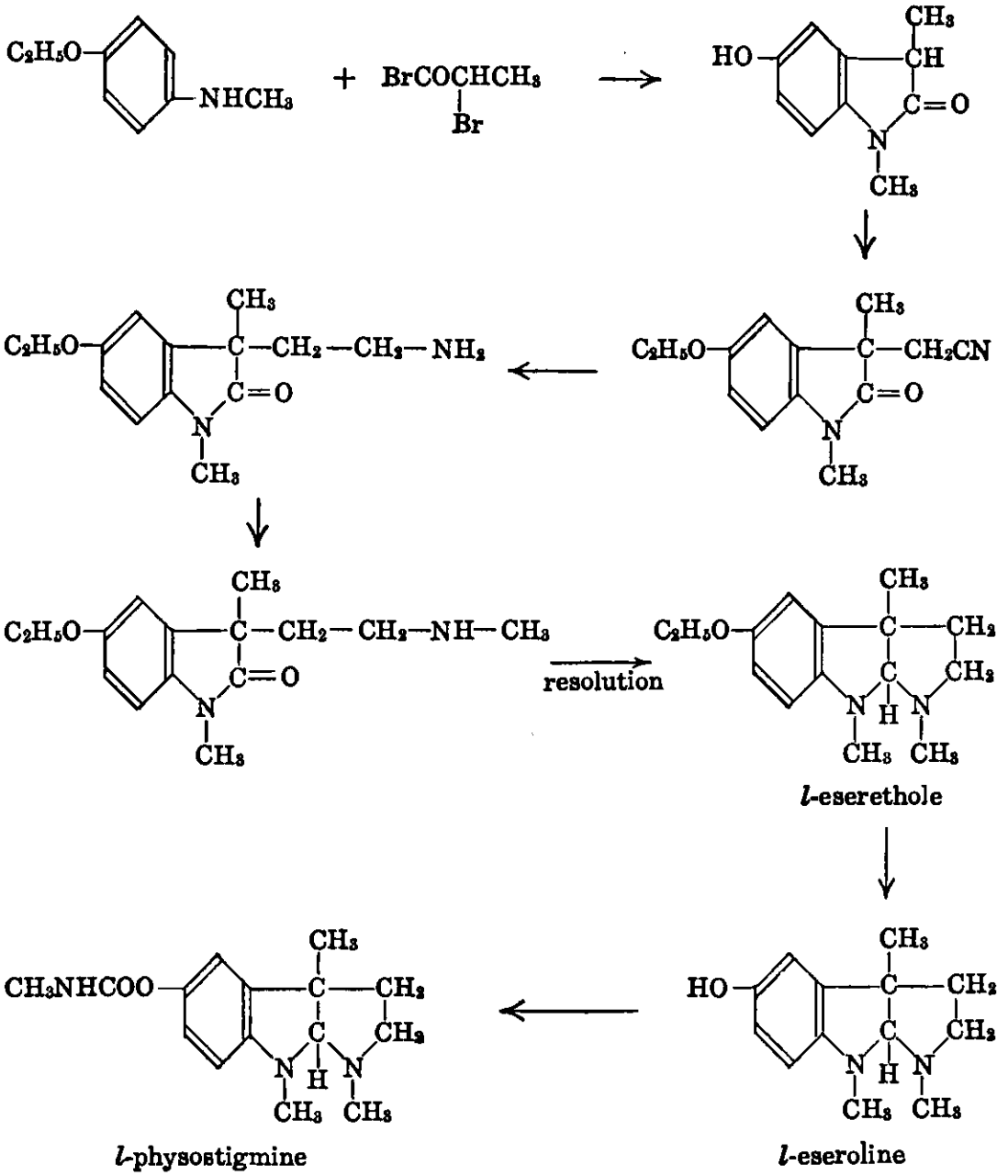


The racemic di-nor-eserethole has also been resolved into its optically active forms,[17] and the latter have been converted into the dextro and laevo nor-eserethole and the corresponding optically active eseretholes.

The complete synthesis of physostigmine has been achieved by Julian and Pikl, who also obtained the following intermediates: *d,l*-desoxy-nor-eseroline,[21] *d,l*-desoxy-eseroline,[22] *d,l*-eserethole,[23] and the laevo forms of eserethole, eseroline, and physostigmine.[24]

N-methyl-para-phenetidine reacted readily with alpha-bromopropionylbromide, followed by treatment with aluminum chloride to effect

ring closure. Re-ethylation was followed by condensation with chloracetonitrile in the presence of sodium ethoxide and catalytic hydrogenation to the corresponding amine. After methylation by Decker's method [25] the resulting secondary amine-ketone was treated with sodium and alcohol to effect reduction and ring closure to *l*-eserethole. Dealkylation with aluminum chloride yielded *l*-eseroline [26] which was treated with methyl isocyanate to yield laevo-physostigmine:



Geneserine, $C_{15}H_{21}O_3N_3$, has been shown to be the N-oxide of physostigmine, and it is, with one exception,[27] the only naturally occurring alkaloid-N-oxide. A number of investigators have examined this

alkaloid,[28] and it is now thought to be identical with a base previously called eseridine.

Eseramine, $C_{16}H_{25}O_3N_4$, can be separated from physostigmine by extraction of the latter from an ethereal solution by means of dilute sulfuric acid, whereby the former will remain in the solvent phase.[29] It is soluble in hot alcohol from which it can be recrystallized (m.p.: 245°).

Physovenine, $C_{14}H_{18}O_3N_2$ (m.p.: 123°), has been found in the mother-liquors of the crystallization of eseramine.[30]

NATURAL SOURCES

Physostigmine is usually prepared from the dried, ripe seeds of *Physostigma venenosum* (Balfour) (family of Papilionacaea). The drug is also known as calabar beans, esere nut, or ordeal bean.

The plant is indigenous to West Africa where it grows wild in the vicinity of the Gulf of Guinea and along streams which flow through the country inhabited by the tribes of Calabar. The ripe fruits often drop into these streams and are then collected by the natives who use them as an ordeal, which usually proves fatal to the accused.

The plant is perennial, has a spreading rhizome with numerous rootlets with small succulent tubers, and a long, climbing, woody stem. The leaves are pinnately compound and stipulate, with each leaf containing three ovate leaflets. The flowers occur in pendulous racemes and bloom almost throughout the year with a pink-purplish color. The fruit is brown, reticulated, compressed legume, containing two or three oblong or reniform seeds which are also of brownish color. The ripe fruit, although found throughout the year, is most abundant from June to September during the rainy season.

The seeds, which were official in the *U. S. Pharmacopœia* (9th Edition) but are not official now, were described there as follows: "Oblong or ellipsoidal, somewhat compressed reniform, from 15 to 30 mm in length and from 10 to 15 mm in thickness; externally reddish to chocolate brown, smooth, somewhat wrinkled near the brownish black groove, the latter being about 2 mm in width and extending almost the entire length of the convex edge and in which are found frequently the remains of the white membranous funiculus, the margins of the seed-coat on both sides of the groove somewhat elevated, of a yellowish-red or brownish-red color and somewhat thickened; embryos large, white, with short hypocotyl and two concavo-convex cotyledons; taste at first starchy afterwards acrid." Further descriptions and methods of assay of the crude drug can be found in the *U. S. Pharmacopœia* (9th Edition), or in the *U. S. Dispensatory* (22nd Edition).

The plant has been cultivated in India and Brazil, with more success

in the latter country. Supplies obtained through London usually come from the Niger and Calabar rivers through Lagos.

The crude drug of commerce is often adulterated with material containing little or no physostigmine. These seeds are usually derived from *Pentaclethra macrophylla* Benth., which seeds are more or less flattened and oval or triangular; *Entada scandens* Benth. or *Entada phaseloides* Merrill, which have brown, shiny, lenticular seeds of about 5 cm width; *Mucuna urens* de Candolle, which seeds resemble *Entada phaseloides* but are somewhat discoid; and finally the seeds of *Physostigma cylindrospermum* Holmes, which are about 4 cm long and can also be distinguished by their shorter grooves.

Only physostigmine salicylate is official in the *U. S. Pharmacopœia* (13th Edition) and in Great Britain, but Lamella of physostigmine is also recognized in the latter.

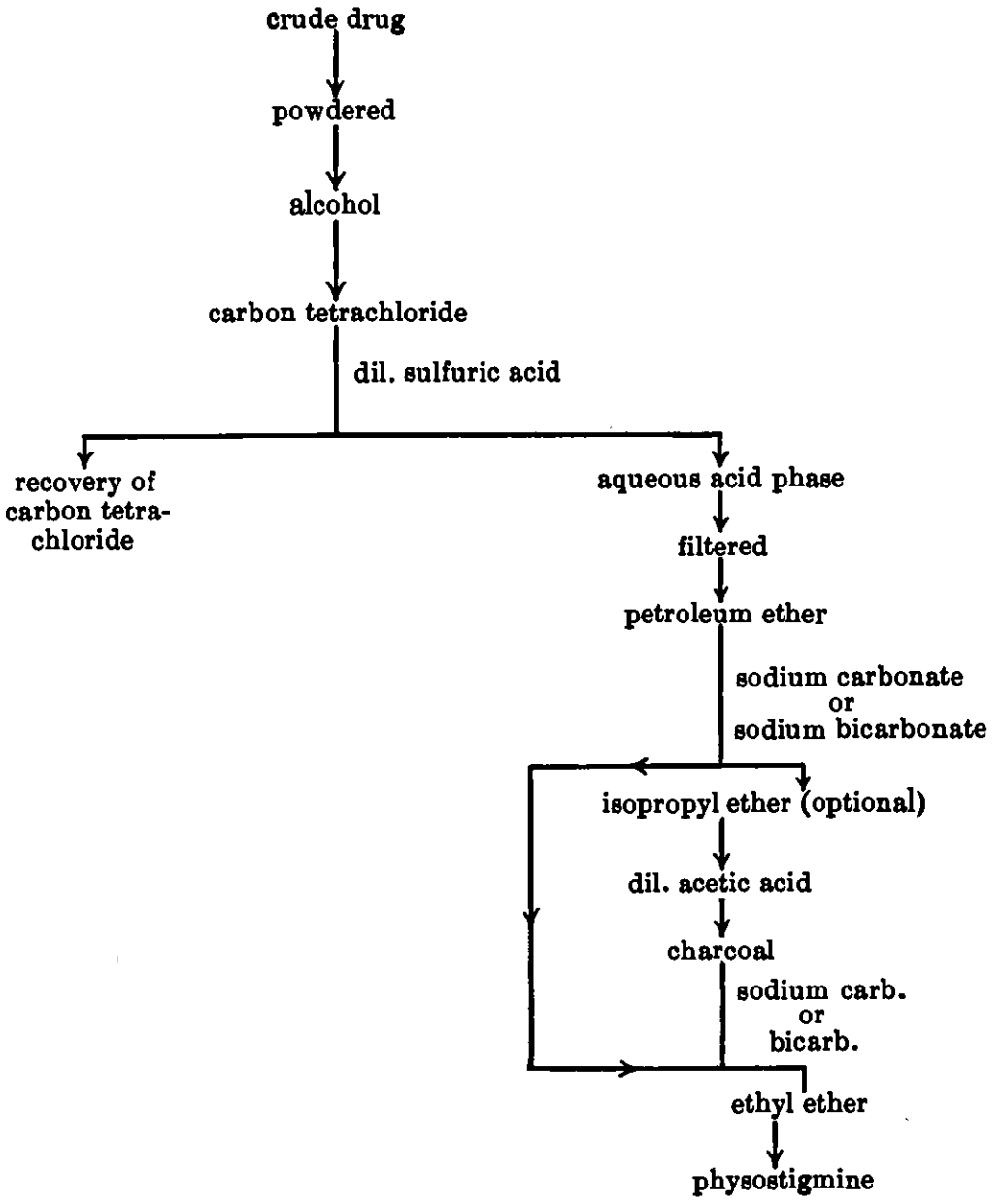
MANUFACTURING METHODS

In a large-scale production of physostigmine, the following method has given excellent results.

The crude drug is reduced to a powder of about 40–60 mesh size and is charged into the extractors. Objections have been raised and precautions have been urged [31] that no metals or metal salts be present during the extraction of physostigmine in order to avoid the formation of hard-to-remove colored by-products, although no such adverse effects were observed by the author. It is possible, however, that the admixture of sodium carbonate solution followed by extraction with ether or another solvent may present a different picture altogether.

The powdered drug is then extracted with moderately warm methanol (45° C) or denatured ethanol which is circulated as described elsewhere. It has not been possible to decide whether a method of countercurrent extraction would be of advantage or of disadvantage. It has been established that heating of concentrated extracts or solutions of physostigmine or its salts should be avoided wherever possible because of the relatively unstable character of this alkaloid. On the other hand, crude, dilute, alcoholic extractions of physostigmine contain the base in the form in which it occurs in the crude drug, possibly a tannate, and therefore might be considerably more stable in that form. In any case, commercial synthetic methanol, which contains only very small amounts of water, is to be preferred to the 90% alcohol usually recommended. The addition of small amounts of tartaric acid to the alcohol prior to extraction was thought to be beneficial by increasing the yield to a certain extent, but this observation could not be confirmed.*

* Private communication from Mr. R. L. Brown.



The combined alcoholic extracts are concentrated under vacuum, and the temperature should be kept near or below 45° C. This is especially important near the end of this operation, when the thick syrupy residue might tend to overheat locally. Continuous stirring is, therefore, indicated.

The residue from this distillation is mixed with carbon tetrachloride or ethylene dichloride (5–10 times its volume), and the resulting solution is then blown into the mixing tank as described previously. The physostigmine is removed from this solution by extraction with dilute sulfuric acid (0.5–1%). Three to four extractions are usually necessary in this phase of the purification. The aqueous acid extractions are com-

bined and are allowed to stand overnight. A small amount of resinous material usually separates and can be removed by decantation, filtration with the addition of filteraid, or by repeated extraction with petroleum ether. The latter has the additional advantage of removing a certain amount of coloring matter from the acid solution and should be used in any case for that purpose.

The clear aqueous extract, which has been further purified by treatment with petroleum ether, is now charged into an extractor equipped with a mechanical stirrer. If the liquid is of a light brown color, extraction of the alkaloid can be undertaken directly with ethyl ether. If the aqueous phase is of a fairly dark, chocolate brown color, further purification is indicated and extraction of the alkaloid is performed with isopropyl ether. The appropriate solvent is added to the dilute acid solution in a sufficient amount to assure extraction of the base. This is usually 10–15% of the volume of the aqueous phase. Stirring is started, and a concentrated solution of sodium carbonate or sodium bicarbonate is added slowly until the liquid shows a strong alkaline reaction. Stirring is continued somewhat longer, and the ether is allowed to separate. The aqueous alkaline layer is carefully removed and is extracted twice more with the same solvent used previously. A sample of the ether, on extraction with dilute hydrochloric acid, should give only a cloudiness with Mayer's reagent.

If further purification of physostigmine is necessary, isopropyl ether is recommended for the extraction. The ether extractions are now combined and are extracted three or four times with a small volume of 2% acetic acid, until all the alkaloid has been removed. These aqueous acid extractions are combined and stirred in the cold with a small amount of charcoal (free of iron and other metals if possible) and filteraid, and are finally filtered after one or two hours. The filtercake is washed with dilute acetic acid. The combined filtrate and washings are then mixed with ethyl ether and the alkaloid is liberated by the addition of sodium carbonate or bicarbonate as described previously, and extraction with ethyl ether is continued until all the physostigmine has been removed. The ethyl ether extract thus obtained corresponds to the one mentioned before (see diagram, p. 230).

These ethyl ether solutions of physostigmine can now be used for the preparation of the free base, the salicylate, the sulfate, or other salts. An additional treatment of this ethereal solution with charcoal and filteraid has been recommended, but this has often been found to be more harmful than beneficial. Unless the charcoal used is of the highest purity and the best grade, impurities may be added to the ethereal solution instead of being removed from it.

The ether solution is carefully dried with anhydrous sodium sulfate, filtered, and the filtercake is washed with some dry ether. The combined clear filtrates are distilled in a glass-lined still under ordinary pressure to a volume of about 4 liters.

This concentrated solution of physostigmine in ether is then removed from the still and is usually used for the direct preparation of the salicylate. The alkaloid or other salts are then manufactured from the salicylate after liberation of the base, thus introducing an additional step of purification. Whether or not this is necessary will depend entirely upon the appearance of the individual batches. If this additional purification is desired, the dried physostigmine salicylate is suspended in water, stirred with ether, and the base is liberated by the addition of a solution of sodium carbonate or bicarbonate. The extraction with ether is repeated, and the combined ether extracts are processed further as described later.

Physostigmine Alkaloid

An ethereal solution of physostigmine is dried carefully with anhydrous sodium sulfate and is then distilled to dryness under vacuum at as low a temperature as possible. The residue, if it shows a slight pinkish color only, can be powdered and used as such. If additional purification is desired, this glassy resinous material is dissolved in a small amount of dry benzol, and the base is crystallized upon the addition of petroleum ether. After filtration it is washed with petroleum ether and dried under vacuum in the dark. Extensive exposure will turn this base and other salts of physostigmine pink or red.

Physostigmine Salicylate

To a dry, ethereal (ethyl or isopropyl) solution of physostigmine is added a dry, ethereal solution of an excess of salicylic acid, and crystallization is induced by scratching and seeding with an authentic crystal of physostigmine salicylate. It is usually impossible to start crystallization without seeding. After allowing to crystallize overnight, the material is filtered and washed well with ether. It can be air-dried. The salicylate has also been obtained from a solution of physostigmine in dilute acetic acid by the addition of a solution of sodium salicylate, but preparation from the ethereal solution is to be preferred.

Physostigmine Sulfate

The preparation of this material is very similar to the preparation of atropine sulfate with the exception that the temperature should be kept even lower to induce and facilitate crystallization of the sulfate.

A known quantity of the dry, glassy physostigmine base as described under **Physostigmine Alkaloid** is dissolved in dry, absolute methanol until a 50–60% solution results. This solution is then cooled thoroughly with ice and salt, and to it is added a cold solution of the calculated amount of sulfuric acid in anhydrous methanol, until the solution is neutral to moist litmus paper. An equal volume of acetone is then added, and the flask is cooled with dry ice. Care should be taken to keep carbon dioxide from entering into the flask. This can easily be done by attaching a reflux condenser or a long glass tube. More acetone is added from time to time until no more physostigmine crystallizes. The material is then filtered on a Buchner funnel which had previously been cooled with dry ice, and the filtrate is washed with acetone and ether. It is deliquescent and must be dried under vacuum in the dark.

Physostigmine Meta-cresotate

This salt is obtained from ethereal solutions of physostigmine and meta-cresol. It is said to be nondeliquescent (m.p.: 160–163°).

Other Salts of Physostigmine

The benzoate, prepared by mixing ethereal solutions of physostigmine and benzoic acid, is said to be nondeliquescent (m.p.: 116°). The sulphite [32] is also said to be a stable salt of physostigmine.

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

Physostigmine, when injected into the bloodstream, is a powerful stimulant of the parasympathetic nerve endings. This action is due to its destructive action on cholinesterase.[33] Among the symptoms are stimulation of the salivary and sweat glands, increased tonus of the intestinal muscles, constriction of the bronchi, and contraction of the pupil of the eye. The last effect is usually obtained by direct introduction into the eye, avoiding all secondary symptoms. Physostigmine increases the output of epinephrine in the body,[34] and this may occasionally inhibit the stimulation of the peristalsis action, which might be indicated in chronic constipation and other deficiencies attributed to the intestinal muscles. It has been successful in the treatment of postoperative flatulence.[35] Physostigmine increases the pulse rate and increases the blood pressure.[36] It is said to have a depressant action on the motor centers in the spinal cord,[37] and because of this effect it is used in tetanus and strychnine poisoning.

Administration of toxic doses of physostigmine causes a general lessening of reflexes, muscular tremors or convulsions, contraction of the

pupil of the eyes, and gradual loss of motor power. Death is due to paralytic asphyxia.

ANALYTICAL METHODS

According to Rosenthaler [38] no microchemical crystalline precipitates are known for the identification of physostigmine, and we are dependent upon its color reactions.

Ammonia gives a precipitate which is soluble in an excess. A solution in nitric acid is yellow and turns red on warming, but a yellow solution in sulfuric acid turns green. Iodic acid yields a purplish-blue color, and hydrochloric acid yields a red color. Diazotized sulfanilic acid yields a red pigment, but mercuric chloride, platinic chloride, and potassium dichromate give precipitates only in a concentrated solution. Precipitates are also obtained with aurichloride, potassium bismuth iodide, phosphomolybdic, picric and tannic acids, as well as with trinitrothymol, trinitrophloroglucine, tetranitrophenolphthalein and hexanitrodiphenylmethane.[39] A color reaction of physostigmine and its salts with glyoxylic acid has been studied intensively by Perez.[40]

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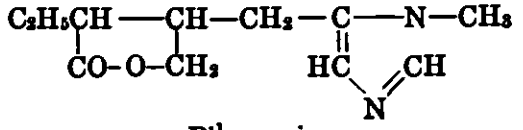
CHAPTER XVI

ALKALOIDS OF PILOCARPUS

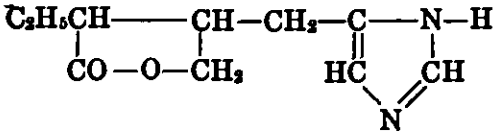
CHEMISTRY

Pilocarpine is the main alkaloid in a number of species of pilocarpus, and it is the one which is physiologically most important.



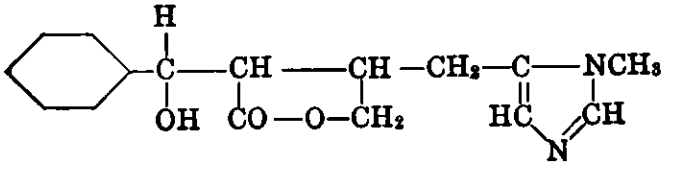
Pilocarpine

Other alkaloids accompanying it are isopilocarpine, an isomer of the base mentioned above, pilocarpidine, which could be regarded as nor-pilocarpine, since it yields the latter alkaloid upon N-methylation together with another isomeride of pilocarpine, neopilocarpine.



Pilocarpidine

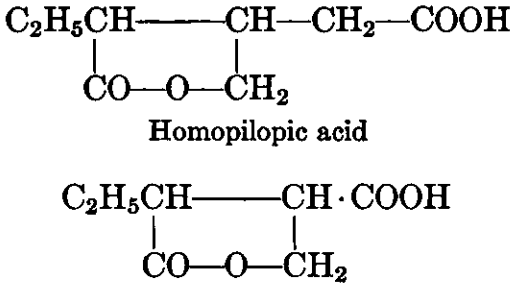
Pilosine has been found to accompany pilocarpine and isopilocarpine in small amounts.



Pseudopilocarpine and pseudojaborine are found in only some of the pilocarpus species, especially *Pilocarpus spicatus*.

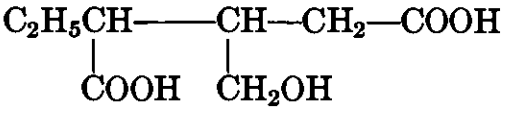
Early investigations were undertaken by Hardy,[1] Gerrard,[2] and others, a good account having been given in 1897 by Petit and Polonovsky.[3] Our present-day knowledge of the constitution of pilocarpine and of the other jaborandi alkaloids is due mainly to the works of Jowett [4] and of Pinner.[5]

When isopilocarpine was subjected to oxidation with permanganate, a mixture of nitrogen-free acids resulted, which could be separated by fractionation of their ethyl esters. Pilopic acid, $C_7H_{10}O_4$ (m.p.: 104°), one of the acids isolated, gave rise to two different barium salts under different experimental conditions, namely, $Ba(C_7H_9O_4)_2$ in the cold, and $BaC_7H_{10}O_5$ when the acid was heated with barium hydroxide. This led to the belief that a hydroxydibasic acid was present in the form of a lactonic acid. Caustic fusion of pilopic acid gave *n*-butyric acid. Homopilopic acid, $C_8H_{12}O_4$ (b.p.: 235°/20 mm), also gave two barium salts, $Ba(C_8H_{11}O_4)_2$ and $BaC_8H_{12}O_5$, and on caustic fusion it gave alpha-ethyl-tricarballylic acid. Pilopic acid was thought to be produced by decarboxylation of homopilopic acid, followed by oxidation of the resulting methyl group. Considering the products of alkaline fusion mentioned previously, and because of the fact that homopilopic acid proved to be very stable and was thought to be a gamma-lactonic acid, the following formulae were suggested for pilopic and homopilopic acid.



Homopilopic acid

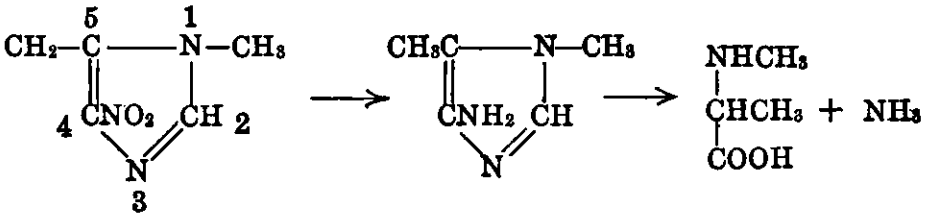
Pilopic acid

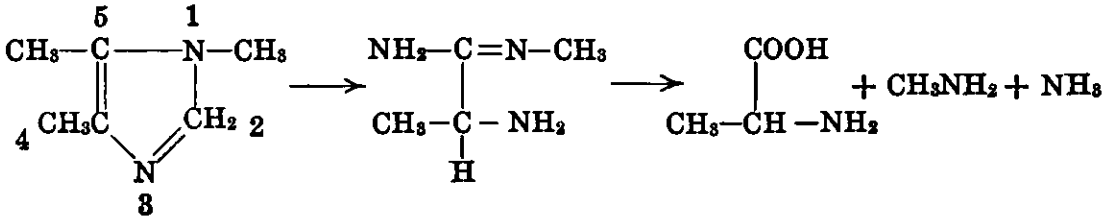


Tricarballylic acid

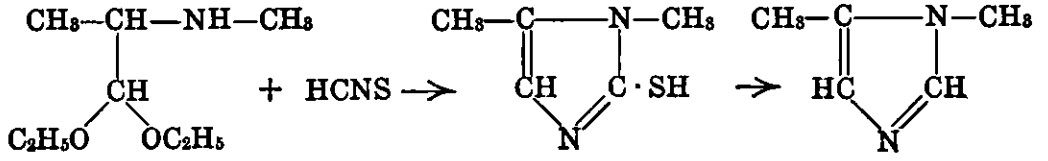
Jowett [6] showed that pilocarpine on oxidation with permanganate also yields homopilopic acid. The two nitrogen atoms present in pilocarpine were shown to be eliminated in the course of the oxidation as ammonia and as methylamine.

These facts and other experimental data accumulated led Pinner and Schwarz [7] to propose a formula for pilocarpine, which was subsequently confirmed by Jowett.[8] The latter prepared a series of disubstituted glyoxalines from isopilocarpine by distillation with soda lime, but was at that time unable to decide whether the 1:4 or the 1:5 derivative was present, and pointed out that either formula could account for the reactions of pilocarpine. Pyman [9] investigated the dimethyl glyoxaline obtained by Jowett and proved it to be the 1:5 derivative (b.p.: 225°), because its nitro-derivative on reduction with stannous chloride gave *d,l*-N-methyl-alanine, whereas the 1:4 isomeride, in a similar series of reactions, gave *d,l*-alanine.





The 1,5-dimethyl glyoxaline was finally synthesized by Burtles *et al.*[10] from alpha-methylaminopropionacetal with thiocyanic acid, followed by oxidation with nitric acid.



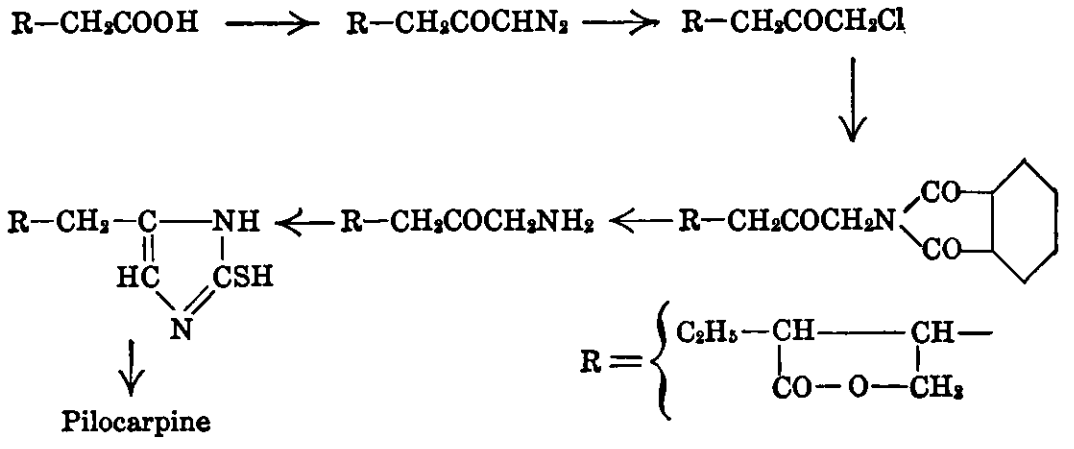
Pinner [11] assumed the difference between pilocarpine and isopilocarpine to be one of structural isomerism, depending upon the point at which the pilopyl residue is attached to the glyoxaline ring, whereas Jowett [12] suggested stereoisomerism as the only difference. He and Langenbeck [13] were able to convert pure isopilocarpine partially into pilocarpine by treatment with alkali. Langenbeck (*loc. cit.*) demonstrated the fact that the two alkaloids yield different methiodides, and on ozonization they yield different methylamides, namely, homopilopic acid methyl amide (m.p.: 104°) and homoisopilopic acid methylamide (m.p.: 53°). It can be assumed, therefore, that the difference between the two alkaloids is due to stereoisomerism in the pilopic acid residue and possibly to a racemization of the asymmetric carbon to which the ethyl group is attached.

This view was confirmed by a series of experiments by Russian scientists with ethyl pilopates or ethyl-2-keto-3-ethyl-tetrahydrofurane-4-carboxylates, which could be separated into two racemic mixtures. A complete report on that complex investigation has been published by Preobrashenski *et al.*,[14] wherein they come to the conclusion that pilocarpine has the "trans" and isopilocarpine the "cis" configuration. They also describe a technical synthesis of pilocarpine.

Starting from ethyl succinate and ethyl formate, after separating the mixture of racemates followed by resolution, a number of acids, one of

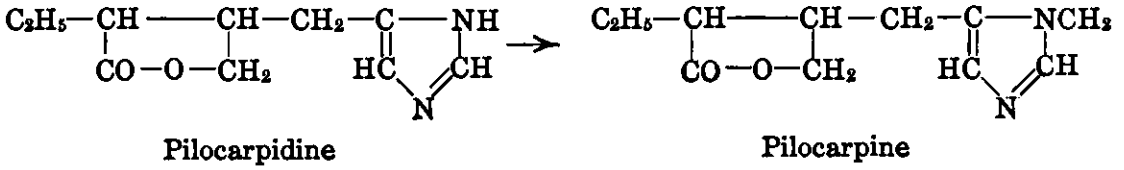
which proved to be identical with an acid obtained by Jowett by permanganate oxidation of isopilocarpine, were obtained. The racemic acids mentioned previously were shown to have a relationship to each other similar to the one of pilocarpine to isopilocarpine.

Pilopic acid was transformed into homopilopic acid, which was then converted into pilocarpine through the following steps: acid chloride, *d*-homopilopic diazo methane, chloromethyl pilopyl ketone, aminomethyl ketone; the last with potassium thiocyanate gave mercapto pilocarpidine, which was converted into pilocarpine by oxidation followed by methylation.



Other syntheses of the alkaloid or of some part of it were described by Dey [15] and by Welch.[16]

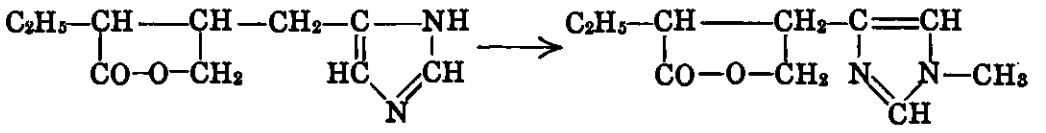
Pilocarpidine was suggested by Harnack [17] to be the imino-base corresponding to pilocarpine, and this was confirmed by Burtles *et al.*,[18] who transformed it into the latter by N-methylation. Spaeth and Kunz [19] obtained isopilocarpidine on treatment with sodium ethoxide, which in turn could be converted into isopilocarpine metho-salts upon quaternary methylation. The relationship of pilocarpidine and isopilocarpidine to pilocarpine and isopilocarpine has been fully confirmed by synthesis as described previously (Preobrashenski, *loc. cit.*).



Pilocarpidine Pilocarpine

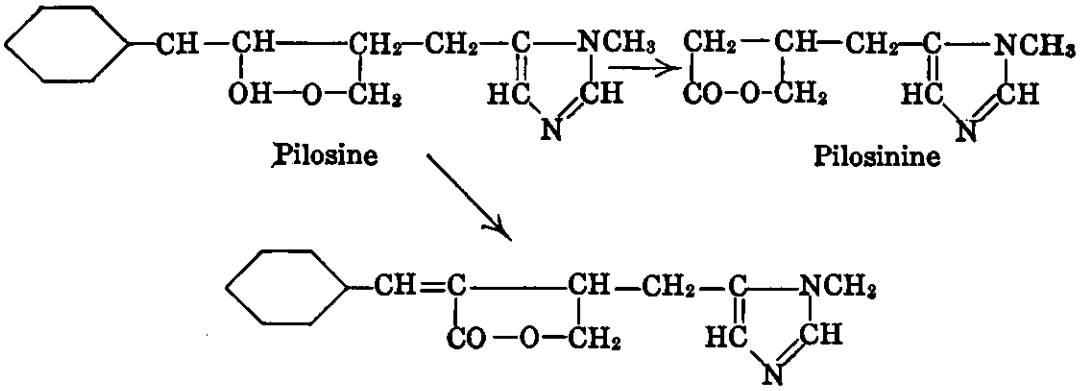
Another base obtained by the N-methylation of pilocarpidine was neopilocarpine (Burtles *et al.*, *loc. cit.*), which is thought to be a structural isomer of pilocarpine. Neopilocarpine can also be isomerized to neo-iso-pilocarpine on treatment with alkali, giving further evidence to the view that the isomerism is due to differences in the pilopic acid part

of the molecule. The difference between pilocarpine and neopilocarpine is due to a different point of attachment between the two rings.



Pilocarpidine Neopilocarpine

Pilosine has been obtained from pilocarpus microphyllus after pilocarpine and isopilocarpine have been removed from the total alkaloid mixture. The base is dextrorotatory in alcoholic solution but laevorotatory in alkaline solution. It contains one methylimino group but does not contain a methoxy group. It behaves as a lactone, and on dehydration with acetic anhydride and glacial acetic acid it yields anhydro-pilosine, which still contains the lactone group. Pilosine splits off benzaldehyde on distillation with potassium hydroxide, yielding a new alkaloid, pilosinine, which is laevorotatory in alkaline solution. Pyman [20] suggested the following formulae for pilosine and pilosinine because of their reactions and because of their physiological properties which are similar to pilocarpine and isopilocarpine. Because pilosine does not isomerize on treatment with alkali, it is thought to be similar to isopilocarpine in its structure rather than to pilocarpine.



Pilosine Pilosinine

Anhydropilosine

Pseudopilocarpine and pseudojaborine have been obtained from *Pilocarpus spicatus* by Petit and Polonovsky.[21] Their structure is uncertain. They are amorphous but yield crystalline salts. Pseudopilocarpine yields a nitrate which crystallizes in needles (m.p.: 142°), and a hydrochloride, prisms melting at 199–201°. Pseudojaborine gives a hydrochloride also crystallizing in prisms (m.p.: 223°), and a nitrate, flakes (m.p.: 158–160°).

The most important data on the other alkaloids have been summarized in Table 16.1.

TABLE 16.1

	Pilocarpine	Isopilo-carpine	Pilocar-pidine	Pilosine	Neopilo-carpine
B.p.	260/5 mm	261/10 mm			
M.p.	34			187	42
Base α_D^{20}	+101	+43	+81.5	+40	
HNO_3	180	160	137		95
HCl	205	128(160)		144	177
HBr	185				
H_2SO_4	134		195	195	
Picrate	161				120
H_2PtCl_6	219		90(187)		
Aurichloride	125	160	125	145	

Pilocarpine is slightly soluble in ether and petroleum ether, but mixes in almost all proportions with water, alcohol, and chloroform. The nitrate crystallizes in prisms, which are somewhat soluble in water (15.6% at 20°), but less so in 96% alcohol (0.7% at 15°) and even less in absolute alcohol (0.37%) (α_D +83°). The hydrochloride (α_D +91.8°) also crystallizes in prisms, sparingly soluble in alcohol but insoluble in acetone. The hydrobromide also forms small prisms. The sulfate is very soluble in alcohol.

The solubilities of isopilocarpine are similar with those of pilocarpine with the exception of the nitrate, which is considerably more soluble in alcohol, thus providing an easy means of separating the two bases.

Pilocarpidine is soluble in water and in most organic solvents. The nitrate crystallizes in prisms (α_D +73.2°) and is very soluble in water and soluble in alcohol. The aurichloride is soluble in water but can be crystallized from acetic acid. The picrate does not crystallize readily.

Pilosine is somewhat soluble in hot water or hot alcohol but sparingly soluble in cold water or alcohol, or boiling ether, acetone, chloroform, and benzene. It is a weak base and does not form salts readily.

NATURAL SOURCES

The drug usually found on the market consists of the dried leaflets of *Pilocarpus jaborandi* Holmes or of *Pilocarpus microphyllus* Stapf. Pilocarpus pennatifolius is no longer available in commerce, but other species appear from time to time, such as *Pilocarpus selloanus* Engler, *Pilocarpus trachylophus* Holmes, and *Pilocarpus spicatus*, also known as "*Aracati jaborandi.*"

All of the pilocarpus species are woody shrubs, indigenous in the tropical and subtropical South American countries and the West Indies, including Guadeloupe, Cuba, Martinique, and other islands. The whole genus has been thoroughly investigated, and many botanical and histological facts have been described by Holmes [22] and by Tunman.[23]

Certain species are peculiar to certain geographic locations and, in commerce, are often named according to their point of origin. *Pilocarpus jaborandi*, also known as *Pilocarpus officinalis* Pohl, is found in the northern and northeastern part of Brazil and is known as *Pernambuco jaborandi*. *Pilocarpus pennatifolius* (*Pilocarpus simplex* Baillon) is found in southern Brazil and Paraguay; hence it is called *Paraguay jaborandi*. *Pilocarpus selloanus* is a variety of the last-named species, and grows a bit further southward, mainly in Paraguay. *Pilocarpus microphyllus*, on the other hand, is found in the northeastern parts of Brazil and is known under the name of *Maranham jaborandi*.

The crude drug is no longer recognized either in the *U. S.* or in the *British Pharmacopœiae*, but a description of the drug is found in the *U. S. Pharmacopœia* (9th Edition):

"The dried leaflets of *Pilocarpus jaborandi* Holmes, known in commerce as *Pernambuco jaborandi*, or of *Pilocarpus microphyllus* Stapf, known in commerce as *Maranham jaborandi* (Fam. Rutaceae) without the presence or admixture of more than 5% of the stalks bearing the leaflets and stems of the same plant, or other matter, and yielding not less than 0.6% of the alkaloids of Pilocarpus.

"*Pernambuco jaborandi:* Leaflets when entire, oval, oblong or elliptical, 4–10.5 cm in length and from 2–4 cm in breadth and with short stout petiolules; summits more or less rounded or acute and emarginate; bases rounded or acute and mostly unequal; margins entire and narrowly revolute, very smooth, shiny, coriaceous and glandular-punctate; upper surfaces grayish to brownish-green, midribs mostly depressed, under surfaces yellowish- or greenish-brown and slightly pubescent on the prominent midvein; peculiarly aromatic when crushed; taste bitterish, becoming somewhat pungent and having a sialagogue effect.

"*Maranham jaborandi:* Leaflets rhomboidally oval to obovate or elliptical, from 1.5–5 cm in length and from 1–3 cm in breadth, the lateral ones nearly sessile, the terminal ones on marginal petiolules, from 0.5–1.5 cm in length; of a nearly uniform grayish or yellowish-green color, rather thin but otherwise resembling *Pernambuco jaborandi.*"

In *Pilocarpus jaborandi* the leaves are two or three jugate and all but the terminal ones have cordate bases, whereas in *Pilocarpus microphyllus* the leaves are alternate and vary considerably in form, the base usually being feebly cordate or unequal, and attenuate.

Pilocarpus pennatifolius (*Paraguay jaborandi*) has grayish-green leaflets, elliptical, obtuse, feebly emarginate, attenuated at the summit and at the base. *Pilocarpus selloanus* Engler seems to be a variety of the aforementioned species, growing in more southern regions and especially abundant in Paraguay. *Pilocarpus spicatus* A. St.-Hilaire is found in Brazil. It has simple leaves, 3–11 cm long and 1–4 cm wide, somewhat tender, dotted, and attached by a short, twisted petiole. They are a pale brownish-green at the lower surface, somewhat darker on the upper side. The leaves of *Guadeloupe jaborandi* are wider and more ovate than those of *Pernambuco jaborandi,* apparently simple or trifoliate, and of deep green color.

The drug of commerce is usually adulterated with one or more species of Pilocarpus, or with leaves of a species of *Swartzia decipiens* Holmes (*Tunatea decipiens* Holmes). The latter can be distinguished by the absence of secreting hairs, and the light and deep green of the lower and upper surface respectively.

MANUFACTURING METHODS

The initial extraction of the crude drug is essentially alike to that described under the solanaceous alkaloids.

Only new and thoroughly dry Pilocarpus should be used for the manufacture of pilocarpine. It has been found that the content of total alkaloids of the crude drug diminishes as much as 50% in the course of one year, even though the drug is being stored in a cool and dry place. Dampness and excessive heat destroys the alkaloids to an even higher extent.

The leaves are usually sold in commerce pressed into bales and it has been found advisable to break up these bales manually prior to milling to remove any foreign material which might jam or break the mill. The leaves are then reduced to a 20–40 mesh powder and are charged into the percolators.

Extraction can be undertaken with either methanol or ethanol. The

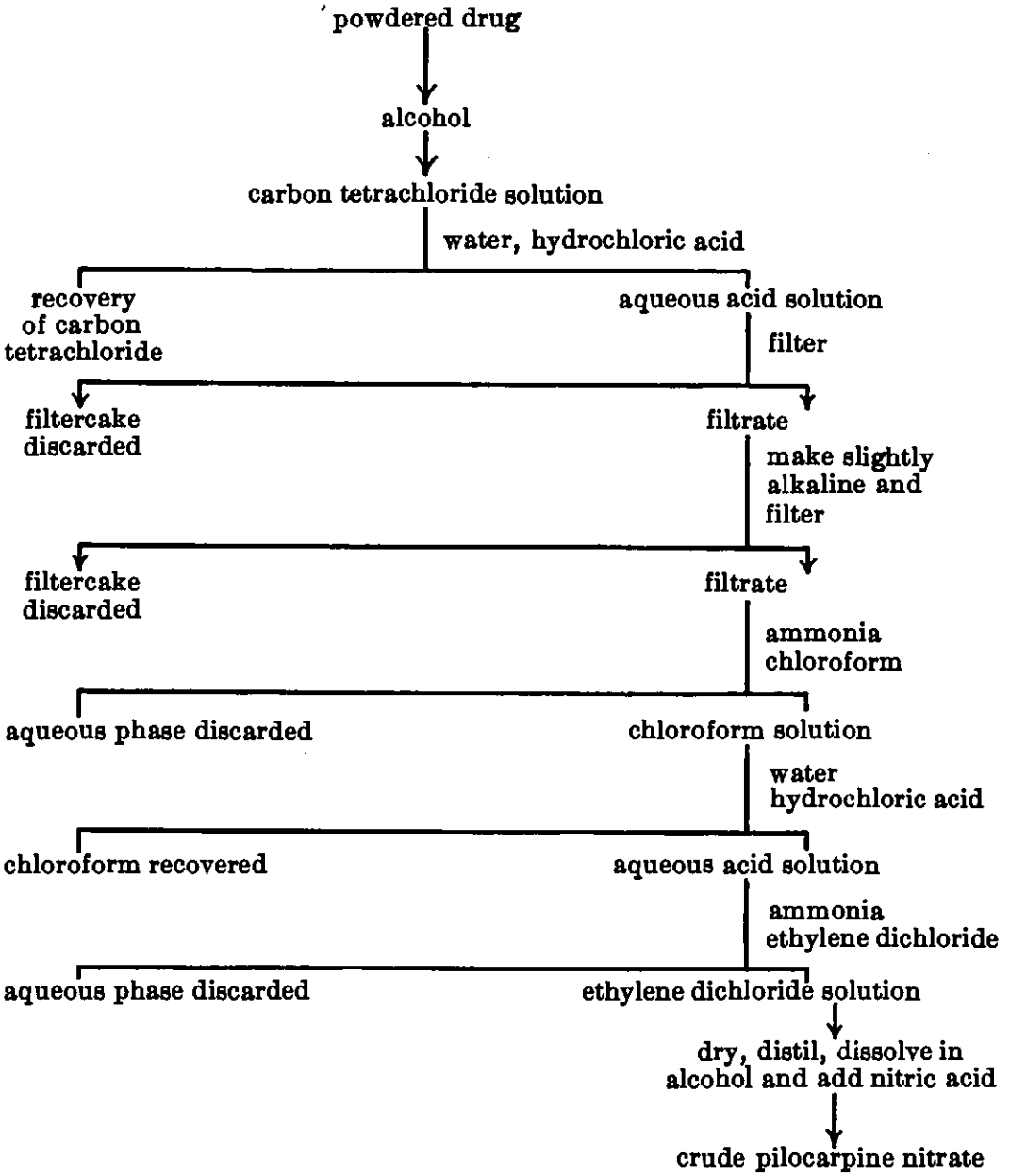
use of acidified alcohol has been found to be superfluous, but warming of the batch during the process, although not absolutely necessary, is of some advantage, as it almost guarantees total extraction of the alkaloids in three extractions. Despite that fact, four extractions in the countercurrent system of extractions are to be preferred.

The extracts are drawn into the still where most of the alcohol can be removed under atmospheric pressure. When the internal temperature of the still rises to about 85°, the rest of the alcohol should be distilled under reduced pressure. When most of it has been removed, the residue remaining in the still is dissolved in ethylene dichloride or in carbon tetrachloride as described in Chapter I. This solution is then transferred to a mixing tank, where there is added to it 2–3 times its volume of warm water (60°) and an excess of hydrochloric acid (about 1 lb of a technical grade of muriatic acid per 1000 lb of drug). After stirring thoroughly for at least ½ hr, the supernatant liquor should be strongly acid to congo test paper. Allow to separate for several hours, draw off the bottom layer which is transferred to another mixing tank where it is extracted again with one half of the amount of water used for the first extraction and a small amount of muriatic acid. A third extraction of the solvent solution is often indicated, which should be performed with an even smaller amount of water and muriatic acid. Separation of the second and third extraction is often made difficult due to the formation of emulsions. These should be removed either by filtering with filteraid, or by adding very small amounts of alcohol cautiously along the side of the vessel and mixing the supernatant liquor ever so lightly by hand. The solvent can now be set aside for recovery when convenient, and the residue can be discarded.

The aqueous acid extracts are combined and are allowed to stand for several hours, preferably overnight, during which time a considerable amount of impurities will be precipitated. The solution is now filtered with filteraid, and the filtercake is washed with acidified water. The clear filtrates are combined and are rendered slightly alkaline by the careful addition of ammonia, until a faint but definite reaction is visible on red litmus paper. This treatment precipitates a fair amount of impurities, and these should be filtered off as soon as possible, to avoid loss of pilocarpine by decomposition. Filtration can be undertaken on a large stoneware filter with the aid of filteraid, or a filterpress can be used, which would be faster, more economical, and more efficient. In either case the filtercake is washed thoroughly with water.

The clear filtrates are combined in a mixing tank, are made strongly alkaline with ammonia, and are then extracted thoroughly with one tenth of its volume of chloroform. Ethylene dichloride has been sub-

stituted at times, but its use at this point of the process cannot be recommended. It has been found that its separation from the aqueous alkaline phase often proved difficult, and that relatively more extractions were needed as compared with chloroform, because of the former's lower solvent power. This offsets the advantage of the lower price and



the fact that a relatively purer extract is obtained, containing fewer impurities as compared with a chloroform extraction. Extraction should be continued with successively smaller portions of chloroform, adding small amounts of ammonia each time, and mixing thoroughly for one half hour each time. Sufficient time should be allowed for settling to

effect a clean separation. Emulsions must be filtered with the aid of filteraid, and the filtercake should be washed with chloroform.

The clear chloroform extractions are combined and are transferred to a mixing tank where the alkaloids are re-extracted with dilute acid, using about one half of the volume of the solvent of 5% hydrochloric acid. Mix thoroughly, allow to settle, and separate. The chloroform is now extracted 2–3 times more with successively smaller amounts of acid, which can also be made more dilute. Three extractions are generally sufficient to remove all the alkaloids from the chloroform, which can now be recovered, and the residue can be discarded. Emulsions which might occur in this series of extractions can be broken by filtration.

The aqueous acid extracts are combined, made alkaline by the addition of an excess of ammonia, and are extracted with small amounts of ethylene dichloride until exhausted of alkaloids. Because of its more selective solubility, ethylene dichloride is to be preferred at this point over chloroform. Although the latter separates somewhat easier because of its higher specific gravity, it also dissolves more impurities and reduces the yield of pilocarpine nitrate on the next step.

The combined ethylene dichloride extractions are combined in a vessel of suitable capacity where they are dried with anhydrous sodium sulfate. Filter on a small stoneware filter, wash thoroughly with ethylene dichloride, and collect the clear filtrates in a suitable still.

This solution of the total Pilocarpus alkaloids should be evaporated under reduced pressure. A vacuum of 25 in. or better is desirable for that operation, to keep the temperature in the still as low as possible to avoid extensive resinification. The last traces of ethylene dichloride can be removed by adding a small amount of dry methanol to the sticky residue under warming and stirring until it is all in solution, and removing the methanol together with the ethylene dichloride under reduced pressure at a low temperature. The gummy residue remaining in the still consists of pilocarpine and other Pilocarpus alkaloids. It should be of dark yellow to light brown color.

The bases are now dissolved in 3–4 times their volume of 95% ethanol. Warming and stirring will be necessary to bring everything into solution.

In this operation, methanol cannot be substituted for ethanol, and alcohol which is less than 95% should not be used. Ethanol which is denatured with methanol cannot be recommended, but ethanol denatured with benzene has been found to be quite efficient. Isopropanol (99%) has also been used successfully.

The alcohol solution is now transferred to a glass or glass-lined container, equipped with a slow but efficient stirrer and an efficient cooling bath. Stirring is commenced, and the solution is cooled with ice and

salt to about 0–5°. Nitric acid (sp. gr. 1.42) is now added slowly through a dropping funnel under continuous stirring. During this addition the temperature should not rise above 5°. Addition of the acid is continued until a slight but definite brown coloration is observed on congo test paper. Crystallization of the pilocarpine nitrate will start while the acid is still being added. Stirring should be continued for some time to make sure that the acid reaction of the solution is permanent. A slight excess of nitric acid should be neutralized with ammonia.

The pilocarpine nitrate is allowed to crystallize in the cold overnight and is then filtered off on a stoneware filter. The filtercake is pressed down well and is thoroughly drained of all alcoholic motherliquors. The first washing is usually undertaken with a small amount of the motherliquor, and subsequent washings are performed with small amounts of acetone. The motherliquor and washings are kept separate and are held for further processing. The crude pilocarpine nitrate can be dried at a moderate temperature (60°).

The acetone washings are combined, and the acetone is recovered. It should not be used again for washing pilocarpine nitrate, as it will contain small amounts of alcohol. The residue from the distillation is added to the alcoholic motherliquor, from which the alcohol is also recovered, after neutralizing the excess acid with ammonia.

The residue contains some pilocarpine nitrate, isopilocarpine nitrate, and small amounts of nitrates of other alkaloids. Some pilocarpine can be obtained from the residue by isomerization of isopilocarpine, but this is of not too great importance commercially. The residue is dissolved in water, the solution is acidified with dilute hydrochloric acid, and it is then treated with a small amount of charcoal in the cold. After filtration, the clear liquor is made alkaline with ammonia and is extracted with ethylene dichloride until exhausted, 4–6 extractions being necessary as a rule. These solvent extractions are combined, and if they appear pure enough, that is, reasonably light in color and free from resinous material, they need not be purified further. If further purification is indicated, the solvent is re-extracted with dilute acid, either hydrochloric or sulfuric, until all the bases have been removed. This aqueous phase is again made alkaline and extracted several times with fresh ethylene dichloride, making certain that an excess of ammonia is always present. The solvent extractions are combined, dried thoroughly with anhydrous sodium sulfate, and are then distilled under vacuum until a syrupy residue remains in the flask. This residue is now subjected to distillation under high vacuum, of 1 mm Hg or better, if possible, at which vacuum a mixture of pilocarpine and isopilocarpine will distil at about 240°. The distillate is dissolved in a small amount

of ethanol, and the nitrate is again prepared as described previously.

The Manufacture of Pilocarpine Nitrate

This salt of pilocarpine, being officially recognized, must meet certain specifications of purity. As a rule, the pure salt can be obtained from crude pilocarpine nitrate in one crystallization.

Twenty pounds of crude pilocarpine nitrate are introduced into the pressure filter (10-gal capacity) and approximately 8 gal of pure methanol are added and the solution is brought to boiling. If a greater amount of crude pilocarpine nitrate has been used, complete solution in the boiling solvent can be achieved by adding small amounts of hot water until solution is complete. The solution is slightly cooled and about 4 oz of charcoal are added, or about 2% of the weight of the crude pilocarpine nitrate used. If the charcoal is added to the boiling solution, sudden foaming and boiling over may result. An amount of filter-aid is then added equal in volume to that of the charcoal used, and, after stirring and digesting the mixture for a few minutes, it is filtered hot through the pressure filter. As the first portion of the filtrate usually contains traces of charcoal which has passed through the paper, it is returned to the vessel for refiltration.

The clear filtrate is placed in a glass or glass-lined vessel of suitable capacity and is allowed to crystallize at room temperature, stirring it frequently for the first few hours to avoid formation of large, hard crystals. After standing at room temperature overnight the batch is filtered on a stoneware filter. It is first washed with a small amount of ice cold methanol and then with a sufficient amount of acetone to remove all the methanol. The finished pilocarpine nitrate is finally placed on glass trays (enamelled trays are not recommended) and is dried at 50°.

The pilocarpine nitrate thus obtained should meet all specifications, but if the crude pilocarpine nitrate used was extremely impure, a second recrystallization might be necessary which is to be undertaken as described previously.

The Manufacture of Pilocarpine Hydrochloride

This salt, which is official in the *National Formulary* (8th Edition), is best made from pilocarpine nitrate which has been recrystallized once. Two methods are possible of converting the nitrate into the hydrochloride. Both methods have been used and both are satisfactory.

Method 1. A sufficient amount of pilocarpine nitrate (taking into account the drop in molecular weight and a 5% loss due to handling) is dissolved in a small amount of 15% ammonia and is extracted at

once with chloroform or ethylene dichloride. The extraction is repeated twice more, adding a small amount of concentrated ammonia each time. After each extraction the solvent phase should be separated from the aqueous phase as carefully as possible to ensure maximum yield and to avoid contamination with inorganic salts.

The solvent extractions are combined and are dried thoroughly with anhydrous sodium sulfate. They are then filtered carefully, and the filtercake is washed with pure solvent which is added to the filtrate. The combined clear filtrates are now distilled, preferably in the laboratory in a large flask, and at as low a temperature as convenient. It has been found that at higher temperatures a certain amount of isomerization to isopilocarpine takes place, with a corresponding drop of the melting point of the final product. For that reason the temperature should be carefully watched near the end of the distillation when the residue becomes thick and syrupy. As pilocarpine hydrochloride is slightly soluble in chloroform or ethylene dichloride, these solvents need not be removed completely. Presence of a trace of these solvents in the next step of manufacture will tend to give better crystals and a nicer looking product, but presence of too much of these solvents might lower the yield.

The residue from the distillation, consisting of pilocarpine base, is now dissolved in ten times the amount of pure acetone and is placed in a glass or glass-lined container, equipped with a glass stirrer of sturdy construction, which need not be too rapid. The flask is now cooled from the outside with ice, and a slow stream of hydrochloric acid gas is introduced under stirring. The hydrochloride of pilocarpine will begin to crystallize almost at once. The temperature in the flask should not be allowed to rise above 10°. The addition of the hydrogen chloride gas should be regulated carefully, and it should be stopped when a slight reaction to congo test paper is reached.

Stirring is continued for another hour, and after cooling for several hours the pilocarpine hydrochloride can be filtered on a stoneware filter. The crystals are thoroughly washed with acetone and are then dried at a moderate temperature.

The purity of this hydrochloride will depend to a large extent on the grade of the pilocarpine nitrate used for its preparation. If it appears necessary to recrystallize the pilocarpine hydrochloride, this can be accomplished by dissolving it in a small amount of dry methanol, filtering the warm solution with charcoal through the pressure filter, evaporating the clear filtrate under vacuum, and crystallizing the hydrochloride by the addition of acetone. If pure pilocarpine nitrate has been used originally, this recrystallization will not be necessary. It is obvi-

ously more advantageous to recrystallize the pilocarpine nitrate instead of the hydrochloride.

Method 2. In this method the distillation of the solution of pilocarpine base and a possible partial isomerization to isopilocarpine is avoided.

Pilocarpine nitrate is dissolved in dilute ammonia and extracted with chloroform or ethylene dichloride as described before. The solvent extractions are combined and are extracted with small amounts of 20% hydrochloric acid. Just enough of the acid is added, in small portions and under very efficient stirring, until a slight acid reaction to congo test paper is visible. The layers of acid and solvent are allowed to separate for an hour or longer, until a clean separation can be effected. The aqueous phase is washed once with ethyl ether. It is then placed in a suitable flask or glass-lined still and is evaporated under vacuum at a low temperature, until it is a fairly thick mixture of syrup and crystals. The still or flask is then opened, and the syrup is poured into ten times its volume of acetone, slowly and under continuous stirring. If concentrated hydrochloric acid is used for the extraction, concentration is not necessary but the thick solution is poured into acetone after having been washed with ether. Rinse with a minimum amount of hot water and add to the acetone liquor. Allow to crystallize in the icebox overnight, filter on a stoneware filter, wash with acetone, and dry at a moderate temperature. If necessary, the pilocarpine hydrochloride can be recrystallized as described before.

The acetone motherliquors from either method can be evaporated and will yield a small amount of pilocarpine hydrochloride, which can be recrystallized and purified in the usual manner.

In both methods for the manufacture of pilocarpine hydrochloride, lower grade and impure pilocarpine nitrate can be used as a starting material, if it is dissolved in a fairly large amount of warm water, enough to yield a 10–15% solution. The solution is then treated with charcoal and filtered warm. One charcoal treatment usually is sufficient to render the solution colorless. It is then made alkaline with ammonia and extracted with a solvent as described before.

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

In its action on the body, pilocarpine is an antagonist of atropine because it stimulates those nerves which are paralyzed by the latter alkaloid. Most glands increase their secretions, solids as well as fluids. Included among these are gastric, lachrymal, and salivary glands. Peristalsis of the intestines is also increased; in fact the muscles of some

organs are caused to contract rhythmically upon injection of pilocarpine. Except by the administration of excessive doses, pilocarpine slightly increases the pulse rate and the blood pressure, but the respiratory center is not affected. Large doses may cause pulmonary edema, and death may be caused by respiratory and circulatory failure.

At the present time its main use in medicine is that of a diaphoretic and sudorific to effect elimination of toxic substances through the skin. As a substitute for physostigmine, by instillation into the eye, it causes the pupil to contract and at the same time the intraocular pressure is lessened. By local application it is said to stimulate the growth of hair, but there exists no definite proof of that. It has been recommended [24] for the treatment of pruritus.

The physiological action of the secondary alkaloids has been studied by Marshall [25] and Pyman.[26] Pilocarpic acid and pilocarpidine are said to be inactive, while isopilocarpine and pilocarpidine resemble pilocarpine in their action, but to a lesser extent.[25] Pilosine and pilosinine are also weaker than pilocarpine in their action.[26]

ANALYTICAL METHODS

A microchemical test for jaborandi leaves has been published by Rosenthaler,[27] whereby the powdered leaves are warmed with ammoniacal chloroform, filtered, and the solvent evaporated. The residue, on shaking with sulfuric acid, hydrogen peroxide, chloroform, and potassium dichromate gives a bluish-violet color.

Ekkert,[28] on treating a solution of a pilocarpine salt with sodium nitroprusside, sodium hydroxide, and hydrochloric acid, obtains a red color. Sodium thiosulfate, when added to part of that solution, gives a green color, while addition of hydrogen peroxide to another part of the solution gives a carmine color.

Pilocarpine gives a colorless solution in concentrated sulfuric acid, but the appearance of a blue color has been reported.[29] Formaldehyde in sulfuric acid gives a yellow solution, which turns reddish brown. Ammonium vanadate in sulfuric acid gives a yellow color which turns green and finally blue. Potassium ferrocyanide gives a yellow color which turns blue. Wagenaar [30] recommends precipitation with aurichloride for the identification of pilocarpine.

Rosenthaler [31] has described a number of microchemical identification tests for pilocarpine: Erdmann's reagent gives square plates and long prisms, also saw-like formations. Reinecke's salts gives bush-like formations of long needles, which are often branched. Trinitroresorcinol gives needles, spears, or long crystals.

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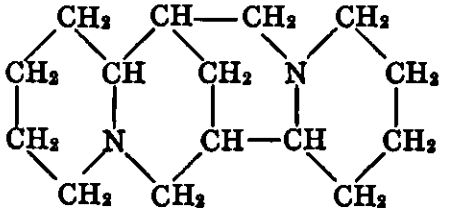
CHAPTER XVII

SPARTEINE

CHEMISTRY

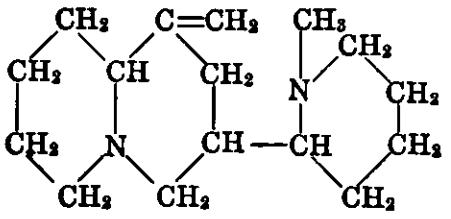
The flowering tops of the common broom produces as its main alkaloid the base sparteine, now known to be identical with lupinidine, which is isolated from the yellow lupin seeds.



Found together with sparteine, but of very little importance, are sarothamnine, genisteine, and ammodendrine.

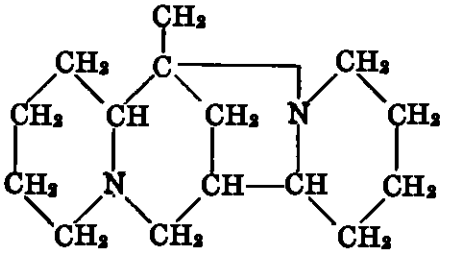
Sparteine was found to have two nitrogen atoms, both tertiary, and neither having a methyl group attached to it.[1] The base seems to be fully saturated as it is not affected by reducing agents or by some oxidizing agents, such as potassium permanganate.

A variety of products have been obtained in the course of the exhaustive methylation and degradation of sparteine. Treatment with methyliodide yields two distinctly different methiodides (α_D −22.75 and α_D −47.2), the first of which, marked by the prefix "alpha," has been synthesized by Schoepf and Braun.[2] Sparteine, upon N-methylation, yields at least two different methyl sparteines, one of which is solid (m.p.: 31°), while the other is liquid (b.p.: 182/16 mm). In addition to these, two more isomers have been obtained, one by Schoepf and Braun [3] and another one by Clemo and Raper.[4]



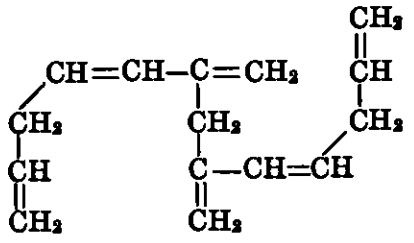
α-Methyl sparteine (?)

Because of the change in structure involved, these methyl sparteines are aptly called de-N-methyl-sparteines. The alpha compound can be converted into isosparteine. This base contains two tertiary nitrogen atoms, none of which is a methyl-imino group.[5]



Isosparteine (?)

Two of the intermediates obtained in the later stages of the exhaustive methylation are hemi-sparteilene [6] and methyl-hemi-sparteilene.[7] Trimethylamine is the second decomposition product formed together with both these compounds. The nitrogen-free hydrocarbon, which is eventually obtained, is sparteilene, $C_{15}H_{20}$.[8]



Sparteilene (?)

Karrer *et al.*[9] reduced the unsaturated compounds obtained as intermediates during the exhaustive methylation of sparteine. Their final product was a hydrocarbon, $C_{15}H_{32}$. Assuming a relationship between lupinine and sparteine, they suggested four possible formulae for sparteine (by adding a piperidine ring to lupinine), and, derived from these, three possible hydrocarbons.[10] All three were synthesized by them, but their physical properties resembled those of the above-mentioned hydrocarbon $C_{15}H_{32}$ so closely that little information was gained from their comparison, except that sparteine should closely resemble one or more of their suggested formulae.

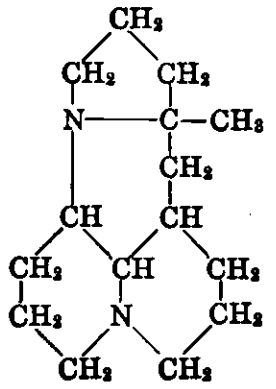
This hydrocarbon, $C_{15}H_{32}$, has recently been identified as 6,8-dimethyltridecane by Schirm and Besendorf,[10a] and its constitution has been confirmed by synthesis. 2-Bromo-heptane was transformed into 2-iodo-heptane, which was reacted with acetoacetic ester and which gave methyl-octyl-ketone after hydrolysis. A Grignard reaction with amyl-magnesium-bromide gave 6,8-dimethyl-8-tridecanol, which was dehydrated and hydrogenated to the desired hydrocarbon.

The oxidative degradation of sparteine yields a number of dehydro-

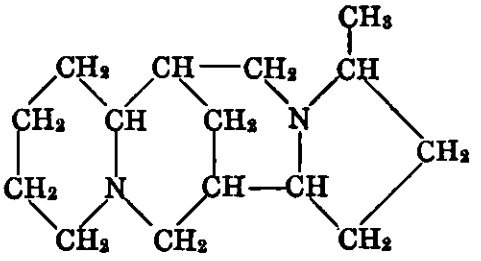
sparteines. Oxysparteine, $C_{15}H_{24}ON_2$, was prepared by Ahrens [11] and by Willstaetter and Marx,[12] and it was thought to be similar to lupanine in containing the oxygen in bridge form, but Clemo and Raper found it to resemble isolupanine.[4] They were not able to reduce it, and the Hofmann degradation was only partially successful, but the latter reaction was achieved by Spaeth and Galinovsky,[13] who obtained hexahydrohemioxy-sparteilene, $C_{15}H_{27}ON$. Spartyrine, $C_{15}H_{24}N_2$, which contains an ethylenic bond, was obtained by Willstaetter and Marx (*loc. cit.*) by the oxidation of sparteine sulfate with chromic acid.

The exhaustive oxidation of sparteine with chromic acid yields to gamma-amino-butyric acid,[14] while under somewhat milder conditions oxalic and succinic acids are obtained.[15] Oxidation with sodium hypobromide leads to a dehydrosparteine,[16] which, on reduction, yields a pseudosparteine (b.p.: 325°), which is isomeric with the known isosparteines. Two isosparteines were obtained as reduction products of alpha- and beta-didehydrosparteines, which were found among the products of oxidation of sparteine with mercuric acetate.[17] The beta-iso-sparteine so obtained is thought to be identical with *d*-sparteine. A dehydrosparteine, which is also obtained during the oxidation mentioned previously, could be reduced to 2-methyl-pyrrolidine.

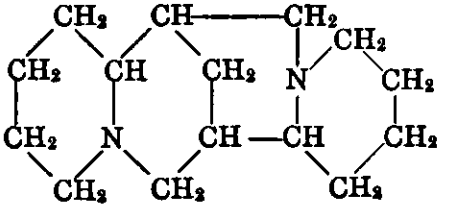
The formation of alpha-methyl-pyrrolidine was an important clue in the elucidation of the formula of sparteine, and proposed formulae for that alkaloid were based on combinations of beta-lupinane and alpha-methyl-pyrrolidine. Winterfeld and Kneuer [18] obtained beta-lupinane, $C_{10}H_{19}N$, by the reduction of racemic lupanine with hydriodic acid, but this experiment could not be confirmed by Clemo *et al.*[19] It was shown, however, that racemic lupanine could be reduced to racemic sparteine,[20] while dextro- and laevo-lupanine were reduced to laevo- and dextro-sparteine respectively.[21] Lupanine differs from sparteine only in having a carbonyl group replaced by a methylene group. This carbonyl group is thought to be present as a lactame. The following formulae for sparteine were suggested by Winterfeld and Kneuer (*loc. cit.*):



Slight modifications in these formulae were suggested when it was found that anagyrine could be reduced to laevo-lupanine, which was identical with tetrahydroanagyrine, $C_{15}H_{24}ON_2$, and to dextro-sparteine (hexahydrodesoxyanagyrine), which differs from natural *d*-sparteine only by a slight difference in the optical rotation.[22]

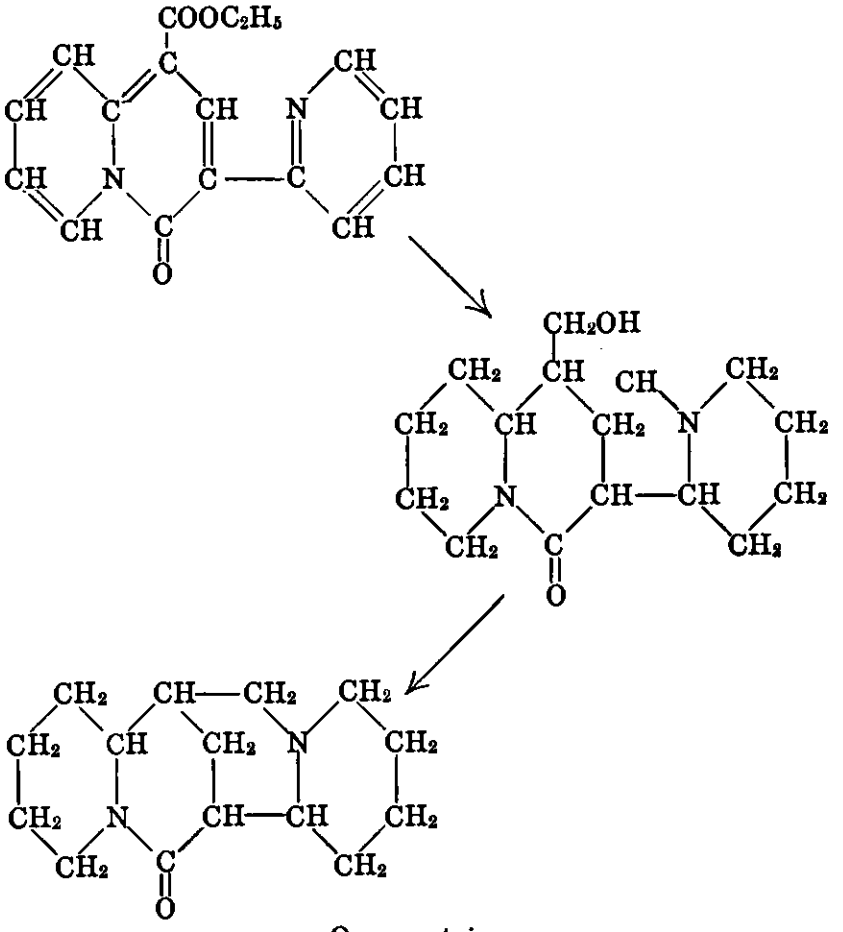


This formula has been further modified by Clemo and Raper[23] to account for the fact that no dimethiodide is obtained from sparteine. This could now be explained by the phenomenon of steric hindrance, if the two nitrogen atoms were brought close together by a suitable arrangement in space.



The structure of sparteine has finally been confirmed by the synthesis of *d,l*-oxy-sparteine, which can be obtained from racemic sparteine by oxidation with potassium ferricyanide. 1-Carbethoxy-4-keto-3-(2′-pyridyl)-pyridocoline was obtained by the condensation of ethyl-pyridyl-2-acetate with ethyl-ortho-formate. Catalytic hydrogenation gave the corresponding octahydropyridocoline, and further reduction with sodium and alcohol reduced the carbethoxy-group to the corresponding carbinol, which however was not isolated. The corresponding 1-bromo-methyl derivative, obtained by the action of phosphorous pentabromide on the carbinol, was again not isolated, but treatment with potassium carbonate in a sealed tube gave the desired *d,l*-oxysparteine.[23]

Sparteine alkaloid is an alkaline, colorless oil, soluble in alcohol, chloroform, and ether, but slightly soluble in water. It is said to be only slightly soluble in benzol, but this could not be confirmed. It is of interest to note that sparteine is monoacidic to litmus and phenolphthalein but diacidic to methylorange. The base has an optical rotation α_D −16.42 in alcohol; its boiling point is 188/18 mm or 327/156 mm. The sulfate, $C_{15}H_{26}N_2 \cdot H_2SO_4 \cdot 5H_2O$, is very soluble in water



Oxysparteine

and alcohol [m.p.: 136° (dry), α_D −22°]. The melting points of other salts are as follows:

Dihydriodide	257–258°
Monoperchlorate	171–172°
Platinichloride	244°
Aurichloride	183–194°
Picrate	208°
Diperchlorate	78° (265 anhydr.)
Oxalate	140°

NATURAL SOURCES

Sparteine is the main alkaloid *Cytisus scoparius* L., but it is also found in a number of other plants, of which at least one belong to a different family than the Papilionaceae, namely, the Papaveraceae.

Scoparius, also known as broom tops, Irish or Scotch broom, is a native of Europe and western Asia, but has been naturalized along the Atlantic coast of the United States. It is a common shrub, and it is

now widely planted as a sand binder. The much-branched bush is 3–10 ft high. Its branches are pentangular, flexible, and of bright green color. The leaves are oblong, trifoliate on the lower parts, and simple on the upper parts of the plant. The numerous bright yellow flowers appear from May to June. The numerous seeds are contained in compressed legumes.

The parts of the plant used for the manufacture of sparteine are the young, green, flexible tops of the branches, which are gathered before the start of the flowering period and dried. These branched twigs are of 1–3 mm in thickness, almost glabrous, of dark green color, and show many patches of brown cork. A description of the microscopic characters of the plant, which was formerly official in both the *U. S.* and the *British Pharmacopœiae*, can be found either there or in the *U. S. Dispensatory* (22nd Edition), page 1565.

The plant has not only been cultivated along the Atlantic coast and the Cape Cod district, but also in the Pacific North West. Most of the drug, however, is imported from Great Britain or Southern Europe. It is usually shipped in burlap bags or in bundles. The drug is sometimes adulterated with the tops of *Osyris alba* Linné. According to Farwell,[24] the latter can be distinguished by its stems which show numerous striations instead of the pentangular appearance. Its leaf buds are at the apex of the angles instead of in the channels between them. Internally the stems are nearly white as compared with the yellowish appearance of Scoparius.

It has been found that the sparteine content of Scoparius varies with the time of harvesting.[25] The amount present rose from 2.3% in March to 3% in May, then fell to 1.25% in June, and rose again to 2.8% in November.

A method for the quantitative estimation of sparteine in broom tops has been published by Wierzchowski.[26]

Sparteine has also been found in the following plants: *Lupinus barbiger* S. Wats.,[27] in *Lupinus laxus* Rydb.,[28] in *Lupinus luteus* Linné, and in *Lupinus niger*,[29] and in *Spartium junceum*, or Spanish broom.[30] It also occurs in *Chelidonium majus* (Fam. Papaveraceae).[31]

THE MANUFACTURE OF SPARTEINE

The first stages of the large-scale preparation of sparteine are very similar to those of ephedrine, and detailed remarks concerning the extraction and concentration will be found there.

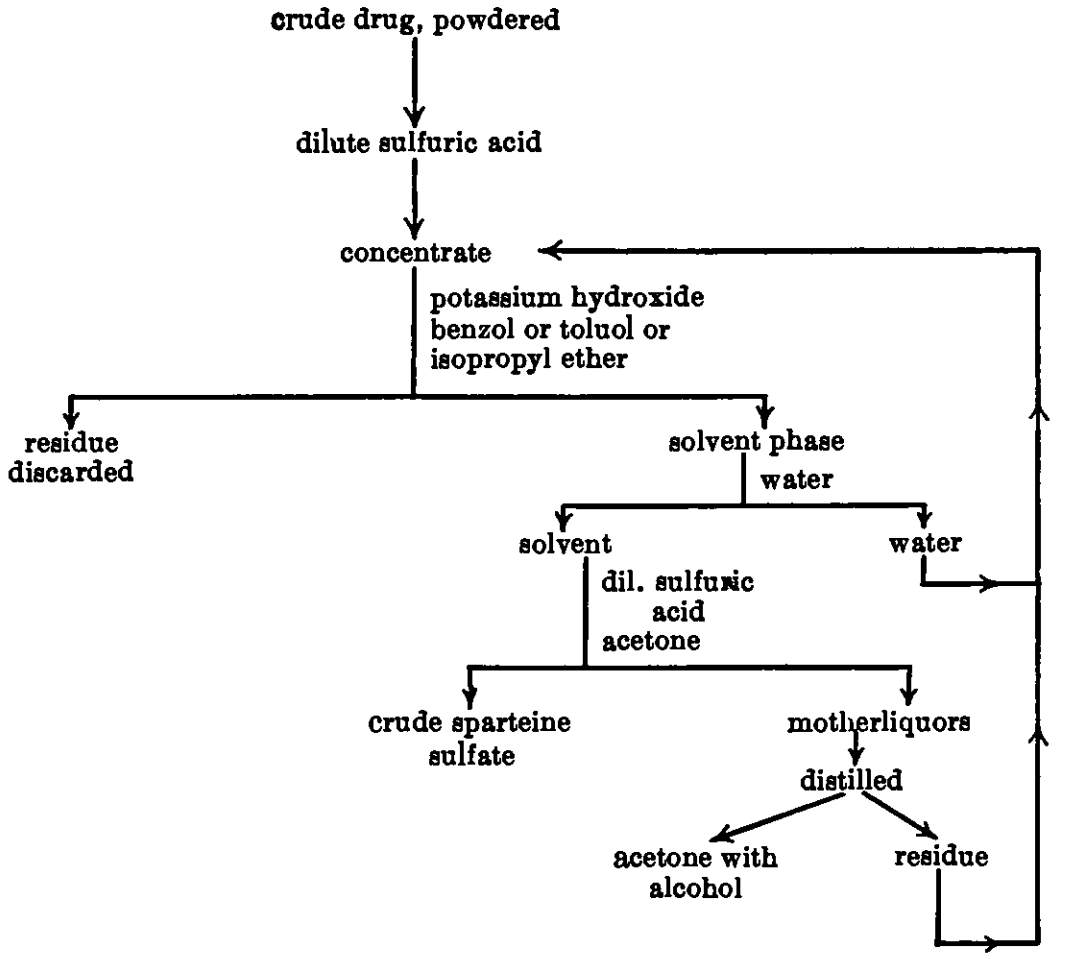
The powdered drug is charged into percolators and is then extracted with dilute sulfuric acid (0.1–0.5%). These extracts should be warmed

and circulated. Three extractions usually remove all the alkaloids from the drug, but under optimum conditions, two extractions have occasionally been found sufficient.

The combined extracts are nearly neutralized by the addition of potassium hydroxide solution to avoid extreme corrosion of the still or other evaporating equipment. They should be slightly acid, however, to avoid loss of alkaloid during the process of concentration.

The combined extracts are then concentrated, first under atmospheric pressure and then under vacuum. Continuous stirring is desirable at all times to prevent caking of the dried extract.

As soon as the combined extracts have been reduced in volume to a thin syrup, the contents of the still are transferred to a cast-iron mixing tank equipped with an efficient stirrer and a bottom outlet. The extract is made strongly alkaline by the addition of a large excess of



potassium hydroxide, and the sparteine is removed by repeated extraction with benzol, toluol, or isopropyl ether. Heating of the batch with live steam or through a jacket facilitates removal of the alkaloid. After the warm batch has been stirred sufficiently and time has been

allowed for settling, the solvent is separated and transferred to a glass-lined mixing tank. It is first washed with a small amount of water to remove inorganic material, and this wash-water is returned to the main batch.

The solvent is then extracted with dilute (15–20%) sulfuric acid. Care should be taken to avoid an excess of sulfuric acid at this stage, because the final liquor, when all the alkaloid has been removed from the extract and transferred to this solution, should be just slightly acid to litmus. The solvent, which is void of sparteine, is returned to the crude extract and is used for further extraction of sparteine. From time to time, more potassium hydroxide should be added to this extract, not only to compensate for the addition of small amounts of wash-water from the subsequent stage of the purification, but also to facilitate the removal of the last traces of sparteine.

The total solution of sparteine in 15% sulfuric acid, which is just acid to litmus, is now concentrated in a glass-lined still under vacuum to a thick syrup. This syrup is now diluted with twice its volume of methanol, and after it has been stirred well it is again reduced to a thick syrup, which is now poured into a large amount of acetone or methyl ethyl ketone (10–15 times its volume). The sparteine sulfate is allowed to crystallize overnight in the cold, and it is then filtered or centrifuged off. It is washed with a small amount of a mixture of 85 parts of acetone with 15 parts of isopropanol, or 80 parts of methyl ethyl ketone with 20 parts of isopropanol. Washing is continued with small amounts of this mixture until the crude sparteine sulfate is nearly white. It is then allowed to dry almost completely on the filter or in the centrifuge. The motherliquors and washings are combined and distilled, and the residue is added to a crude extract prior to or during extraction.

The crude sparteine sulfate is dissolved in five times its weight of alcohol. Either methanol, denatured ethanol, or isopropanol can be used at this stage. The solution is warmed, not only to dissolve all the sparteine sulfate, but also to reduce the viscosity of the solution. After treating with a small amount of charcoal and filtered in the pressure filter, the clear filtrate and washings are combined and evaporated to a syrup under vacuum. To this syrup is added enough distilled water to permit formation of the pentahydrate of sparteine sulfate, which will crystallize upon the addition of acetone. The crystals are filtered and washed with acetone. The salt is official in the *National Formulary* (8th Edition), where its description and specifications can be found.

Sparteine is only rarely used in the form of its base, which can be prepared by dissolving the pure sulfate in a very small amount of water,

from which the alkaloid can be extracted after the addition of an excess of potassium hydroxide. After drying of the ether with anhydrous potassium carbonate, it is distilled and the base is purified by distillation under vacuum.

Sparteine camphor sulfonate is prepared by neutralizing an ethereal solution of sparteine with a concentrated solution of camphor sulfonic acid in methanol. An excess of camphor sulfonic acid can be removed in the subsequent filtration by washing the precipitate with ether.

The preparation of camphor sulfonic acid can best be undertaken as follows: 22 lb of concentrated sulfuric acid are poured slowly into 45 lb of acetic anhydride with stirring and cooling to keep the temperature as low as possible. To this cool mixture is then added 33 lb of natural camphor, and the sulfonation is allowed to proceed at ordinary temperature for several days. The camphor sulfonic acid is then filtered or centrifuged and the crystalline precipitate is washed with ether until all the acetic anhydride has been removed.

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

The action of sparteine resembles that of coniine to some extent. The former appears to have little effect in the central nervous system, but on intravenous injection it decreases both the amount and frequency of the contraction of the cardiac muscles. It has even been able to stop fibrillation of the heart.[32] Sparteine has been used as a substitute for digitalis. In large doses it is also said to act as a diuretic, but this action is more pronounced when Scoparius is administered as such and the effects are probably due to a neutral substance, scoparine. Upon local application it increases the magnitude of the contractions of the uterus, and it has been used in the early stages of labor. In large doses it shows a curare-like action by paralyzing the end-plates of the motor nerves. However, sparteine is mainly used today to combat cardiac irregularities by depressing the action of the muscle fibers instead of stimulating the vagus.

ANALYTICAL METHODS

Sparteine gives no coloration with concentrated sulfuric, hydrochloric, and nitric acid.

When the base is dissolved in dry ether, and hydrogen sulfide is introduced in the presence of powdered sulfur, a voluminous red precipitate is produced.[33] Atropine gives a yellow precipitate and coniine an orange turbidity under the same conditions. The red precipitate produced with sparteine is soluble in water.

Sparteine gives white precipitates with cadmium iodide, sodium phosphomolybdate, and Mayer's reagent, a yellow precipitate with platinic chloride, and green precipitates or colorations with copper salts. A crystalline precipitate is also obtained with potassium ferricyanide.[34]

Microchemical identification tests have been developed by Tunmann.[35]

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CHAPTER XVIII

THE SOLANACEOUS ALKALOIDS

CHEMISTRY

The alkaloids of this group occur in the plant mostly in the form of their oxalates or malates, and because of their structure are often referred to as the "tropane"-alkaloids, the alcoholic part of the ester being a derivative of tropane. Due to their property of dilating the pupil of the eye, they are also called "mydriatic alkaloids."

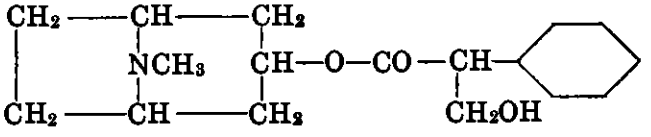
They can roughly be classified into two groups, the esters of tropine and the esters of scopine (oscine). Among the first group are:

Hyoscyamine and its racemate, atropine, $C_{17}H_{23}O_3N$
Apo-atropine and belladonnine, $C_{17}H_{21}O_2N$
Nor-hyoscyamine, $C_{16}H_{21}O_3N$

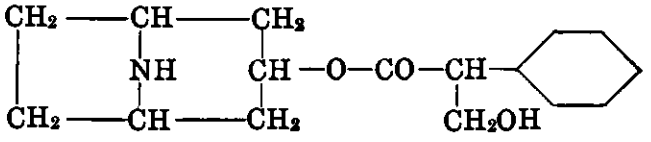
The most important alkaloids belonging to the second group are:

Scopolamine (hyoscine) and its racemate, atroscine, $C_{17}H_{21}O_4N$
Meteloidine, $C_{13}H_{21}O_4N$

Hyoscyamine is the ester of *l*-tropic acid and the basic alcohol tropine,

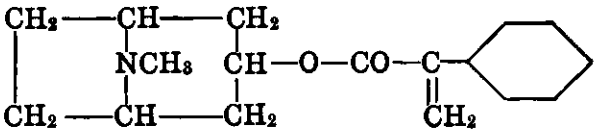


whereas in nor-hyoscyamine the N-methyl group is missing:



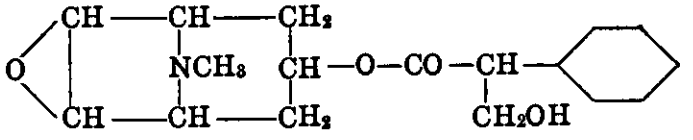
Depending upon the method of hydrolysis, whether under acid or alkaline condition, either tropic acid or atropic acid is obtained, the latter by dehydration with strong acids. Atropic acid is also found in nature as the acid component of apo-atropine and its stereoisomer,

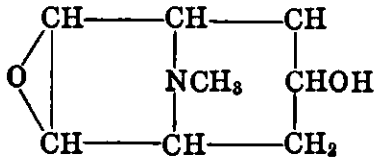
belladonnine:



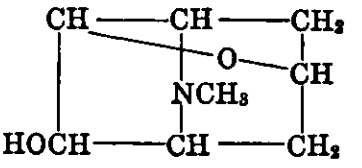
Dehydration can also occur when atropine or hyoscyamine are heated above their melting points at any length of time.

Scopolamine, or hyoscine, as it is often called, can be hydrolyzed by pancreatic lipase in the presence of ammonium chloride into scopine and tropic acid. Scopine is highly unstable and rearranges easily into scopoline, which is also the usual product of either acid or alkaline hydrolysis of scopolamine.



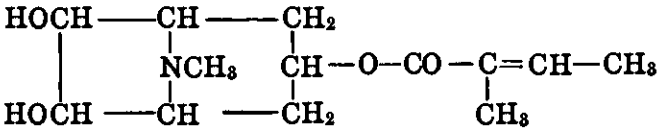


Scopine



Scopoline

Meteloidine is probably an ester of tiglic acid and teloidine having the following structure:



Physical properties of the alkaloids and their components will be found in Tables 18.1 and 18.2.

Although atropine was discovered in 1809 and recognized as an alkaloid by Brandes in 1819, it was not until 1880 that part of it, tropic acid, was synthesized by Ladenburg *et al.* from acetophenone,[1] other syntheses along similar lines being reported later by Mueller [2] and by Wislicenus and Bilhueber.[3] Interesting is the synthesis of tropic acid by reducing ethyl formyl phenyl acetate with aluminum amalgam to form ethyl tropate:

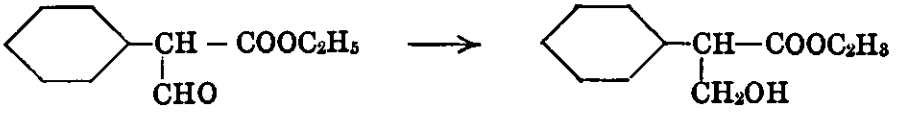


TABLE 18.1

	Tropic Acid	Atropic Acid	Tropine	Scopine	Scopoline	Teloidine
M.p.	129°	107°	63°	76°	110°	170°
B.p.		267°	233°		243°	
HCl						>315°
HBr						295°
Chloroplatinate			200°	219°	203°	
Picrate				231°		
Chloroaurate			212°	216°	220°	½H_2O 225°
α_D	−81.2°					

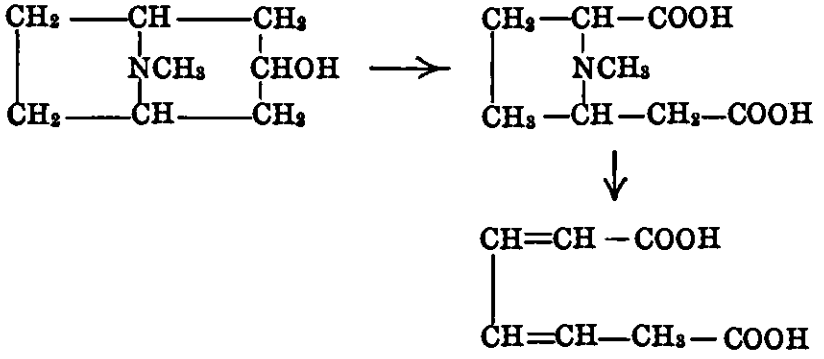
TABLE 18.2

	Hyoscyamine	Atropine	nor-Atropine	Scopolamine	Atroacine	Apo-atropine	Meteloidine	nor-Hyoscyamine
Base	109°	118°	73° 114°	59°	38° 83°	62°	142°	142°
HCl		162°	193°			237°		207°
H_2SO_4	209°	194°	257°					289°
HBr	152°	164°		195°	186°		anhydr. 250°	
Chloroaurate	166°	140°	157°	215°	215°	110°	½H_2O 150°	180°
Picrate	165°	177°		192°	192°		180°	220°
Chloroplatinate	206°	208°						
Oxalate	178°	198°						247°
α_D^{15} of base	50% alcohol −22°			−33°				−23°

Another synthesis was suggested by Mackenzie and Wood,[4] who proceeded from acetophenonecyanohydrine by way of atrolactic acid, atropic acid, and beta-chlorohydratropic acid. The tropic acid so obtained was resolved by King [5] into its optically active isomers.

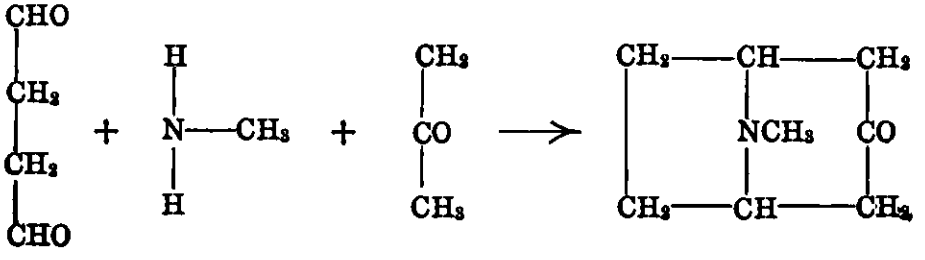
The structure of the acid component of the atropine molecule having been definitely established by its synthesis and resolution, the constitution of tropine, the alcoholic component, was still needed to complete the picture. A pseudosynthesis of atropine by heating together tropic acid and tropine was effected by Ladenburg [6] in 1880, but the structure of tropine was only derived at by Willstaetter [7] in 1898, after a thorough study of the decomposition and oxidation products of tropine.

Some of the compounds under investigation were the dehydration product of tropine, tropidine, $C_8H_{13}N$ (b.p.: 162°),[8] its oxidation product tropinone, $C_8H_{13}ON$ (m.p.: 41°),[9] tropigenine, $C_7H_{13}ON$ (m.p.: 161°),[10] and many others, the final proof of the structure of tropine being offered by Willstaetter [11] when he applied Hofmann's reaction to tropinic acid, obtaining eventually trimethylamine, methyl alcohol, and piperylene dicarboxylic acid:



Final proof of the structure was obtained by the synthesis of tropine, pseudotropine and tropidine from suberone, a heptacyclic ketone.[12]

Robinson, using a simpler method than Willstaetter, obtained tropinone directly [13] in aqueous solution by condensing succindialdehyde, methylamine, and acetone under approximately physiological conditions.

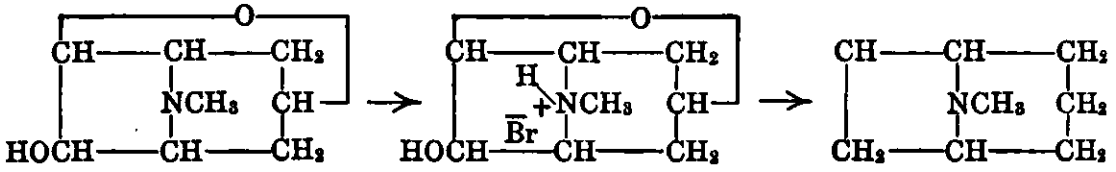


Yields were subsequently improved by the use of calcium acetonedicarboxylic acid instead of acetone,[14] and a number of patents were issued

based on that procedure.[15] Schoepf and Lehmann [16] have shown that the pH of the condensation mixture greatly affects the yield, tropinone dicarboxylic acid being formed at pH 13, but tropinone being obtained in buffered solutions at pH ranging from 3–11, in yields of from 47–86%.

The structure of atropine and hyoscyamine was thus confirmed by its complete synthesis, the optical activity of the hyoscyamine being due to that of the tropic acid, tropine being optically inactive. This principle holds true also for scopolamine. Its alcoholic component is scopoline, optically inactive when isolated by alkaline hydrolysis. It was first examined by Hesse [17] and by Luboldt,[18] and it has been resolved into its optically active components by crystallization of its acid tartrates.[19]

The relationship of scopoline to tropine was established by the formation of hydrobromoscopoline hydrobromide through the action of hydrobromic acid on oscine,[20] reduction of that compound to dihydroscopoline and finally to the hydrocarbon tropane by Hess.[21]



Gadamer and Hammer used the Hofmann degradation to explain the position of the oxygen bridge,[22] which is in a symmetrical position in the scopolamine molecule, making the alcohol scopine optically inactive. Rearrangement to the optically active scopoline takes place upon hydrolysis.

NATURAL SOURCES

Although the solanaceous alkaloids occur in a large variety of plants, and most of them are usually present, certain species can be used for the manufacture of only certain alkaloids, those which predominate, because separation of the different alkaloids on a commercial scale is rather cumbersome and tedious. Hyoscyamine and the alkaloids of its group occur mainly in the species of belladonna and henbane, while scopolamine is found predominantly in *Datura metel*. Ratio of the alkaloids not only differs from species to species, but seems to depend also upon the part of the plant, the location or origin of the plant, and finally its age. Averages will be found in Table 18.3.

The most important raw materials are species of belladonna, henbane, stramonium, and datura, not only because they are most economical, but some of them also enjoy use by themselves, either in powdered form or as starting material for galenical preparations.

TABLE 18.3

Species	Parts	Contents	Percentage	Average
Atropa belladonna	whole plant	hyoscyamine	0.2 –1.1	
	leaves	hyoscyamine	0.15–0.6	0.4
	roots	hyoscyamine	0.1 –0.7	(0.5)
Datura stramonium	leaves	hyoscyamine and scopolamine	0.2 –0.45	
	roots	hyoscyamine and scopolamine	0.21–0.25	
	seeds	hyoscyamine and scopolamine	0.2 –0.5	
Datura metel	leaves	scopolamine	0.25–0.55	
	roots	scopolamine	0.1 –0.22	
Datura arborea	leaves	scopolamine		0.44
Datura tatula	leaves	hyoscyamine and scopolamine	0.4 –0.6	
Hyoscyamus muticus	leaves	hyoscyamine		1.4
	stems	hyoscyamine		0.6
Hyoscyamus niger	leaves	hyoscyamine	0.05–0.1	

Atropa Belladonna

Both the leaves and root are official in the *British Pharmacopœia* but only the leaves are official in the *U. S. Pharmacopœia,* while the roots are now in the *National Formulary.* The plant is an herbaceous perennial. The root is creeping and fleshy in nature. From it rise branching stems, to a height of about 3 ft, which are round and purplish in color. The leaves are attached to the stems by short footstalks. They alternate, are ovate in shape and have a pointed end, and their length varies from 3–8 in. They are almost free from hairs, their underside being slightly paler than their dark green upperside, the underside exhibiting a prominent midrib. The flowers are large and bell-shaped, of a brownish-purple color. The fruit is a berry with a longitudinal furrow on each side, changing from green to deep purple and black, and containing two loculi with numerous seeds and a violet-colored juice.

Atropa belladonna is a native of Southern and Central Europe. It

flowers in June and July, in shady places and along walls, the fruits usually ripening in September. The leaves are usually collected at the time of flowering; the roots are collected in the fall and are taken from plants two or more years old.

The plant has been cultivated in England, the United States, and France, but imports come mainly from both wild and cultivated plants from Yugoslavia, Italy, and Russia. Because of its low market value, the drug is not grown in the United States when it can be imported from abroad. It grows best in the same kind of soil that will produce a good crop of vegetables, lime-containing moist loam. In the United States, the climate of the North-Central and of the North-Eastern states is most favorable; in England it has been grown in Bedfordshire, Suffolk, and in other parts. The plant can probably be adapted to the climate of California and other Pacific states, where the humidity is fairly high and temperatures are not excessive.

Seeds can be sown in a cool greenhouse in midwinter in boxes or pots, or in early spring in cold-frames. After several weeks, seedlings are obtained which can be transplanted to the field, 20 in. apart in each row, the individual rows being two or more feet apart. One ounce of seeds is enough to plant one acre containing about 10,000 plants. After the first year, two crops can usually be obtained each year, the roots being gathered every four or five years to make room for new seedlings.

The herb, consisting of leaves, flowers, and small stems should be dried in the shade, although it has been reported that the manner of drying [23] does not appreciably alter the content of total alkaloids in Belladonna and Hyoscyamus, the Datura species being an exception.[24] Six to seven hundred pounds of dried herb per acre should be obtained under favorable condition each year. The yield of roots after the third year should be 250–300 lb per acre.

It was found that the concentration of hyoscyamine in all parts of the plant of *Atropa belladonna* reaches a maximum in July, but it has also been reported that the time of year at which the herb is collected does not appreciably affect the content of total alkaloids.

According to the *U. S. Pharmacopœia*, "Belladonna leaf yields not less than 0.3% of the alkaloids of belladonna leaf. It contains not more than 3% of belladonna stems over 10 mm in diameter, and yields not more than 3% of acid-insoluble ash." The *National Formulary* states, "Belladonna Root yields not less than 0.45% of the alkaloids of belladonna root. It contains not more than 10% of its stem bases and woody crowns, not more than 2% of other foreign organic matter, and yields not more than 4% of acid insoluble ash." The *British Pharmacopœia* permits the use of *Atropa lutessens* or Indian belladonna leaves with an

alkaloid content of not less than 0.15%, and the *Atropa lutessens* root with an alkaloid content of not less than 0.25%.

Hyoscyamus, or Henbane

The *U. S. Pharmacopœia* states that: "Hyoscyamus is the dried leaf, with or without the tops, of *Hyoscyamus niger* L." This plant, which is usually called henbane, is one of a great variety of Hyoscyamus species, most of which can be found in Europe, northern Africa (Egypt), Asia, and the northern and eastern sections of the United States.

The plant is either annual or biennial. In the first variety the stem is less dawny and has smaller leaves than the biennial plant has. The biennial variety flowers in May or June, whereas the annual plant flowers in July or August. Henbane grows long, tapering, branching, fleshy roots, which have sometimes been mistaken for parsley. The branching erect stem grows to a height of 4 ft. The leaves are alternate, oblong-ovate, soft, with the margins deeply indented (pinnatified). The flowers are yellow, with purple veins, hanging downward. The seeds are small, wrinkled, and almost kidney-shaped, of a grayish color.

Most of the drug is imported from Egypt, although considerable quantities are also harvested in Hungary, Russia, and Belgium. Much of the Egyptian drug seems to be *Hyoscyamus muticus* L., which can easily be detected by the presence of branching nonglandular hairs on both stems and leaves.[25] This variety contains a somewhat larger amount of total alkaloids than *Hyoscyamus niger*. Both varieties have been cultivated in the United States in increasing amounts in recent years.[26]

Hyoscyamus, according to the *U. S. Pharmacopœia*, "yields not less than 0.040% of the alkaloids of Hyoscyamus, contains not more than 25% of its stems, none of which is 7 mm in thickness. It yields not more than 12% of acid insoluble ash." The presence of two alkaloids, hyoscyamine and hyoscine (scopolamine), was demonstrated in 1880 by Ladenburg. It was further shown by Klan,[27] that the relative proportions of the two alkaloids depend a great deal upon the age of the plant. In older plants hyoscyamine predominates.

Stramonium

The dried leaf and the flowering top of both *Datura stramonium* and *Datura tatula* are official in the *U. S. Pharmacopœia*, and should yield "not less than 0.25% of the alkaloids of Stramonium." "It contains not more than 3% of stems over 8 mm in diameter, and yields not more than 4% of acid-insoluble ash." The *British Pharmacopœia* requires the same amount of total alkaloids, but permits "not more than 2%

of other organic matter, not more than 20% of its stem, not more than 1% of the stem being greater than 4 mm in diameter."

The plant grows abundantly in North and South America, in southern Russia and in Asia, but it is also being cultivated to assure a drug of uniform quality. It usually grows near dung-heaps and near deposits of refuse, where rank soil is created. According to the latitude, it may flower from May until August.

Stramonium is an annual plant from 3–6 ft in height. The root is large and has numerous rootlets. The round stem is smooth and spreads into many branches. The leaves are 5–6 in. long, petiolate, toothed at the edges with irregular deep indentations. The flowers are white or purple, and solitary. The fruit is large, contains four locules with numerous flattened seeds, attached to a central placenta.

Also recognized in the *British Pharmacopœia* is the purple stramonium, *Datura tatula* L., which originated in Central America but is now widely distributed both in America and Europe. It differs from the thorn-apple (*Datura stramonium*) by having darker leaves with purplish veins and purple stems and flowers, the difference in color being explained by the presence of a recessive gene.

Stramonium seeds are small, have flattened wrinkled sides, and are kidney-shaped, dark brown to almost black. Their action on the nervous system is said to be more pronounced than that of the leaves.

According to Dohme [28] the roots contain the least and the stems the most amount of alkaloids, with leaves and seeds being in between in their value. There was also reported a difference in the alkaloid content of leaves picked at different times of the day, and of leaves dried at different temperatures and speeds.[28]

The commercial drug is usually collected from wild-growing plants at the time of flowering, in the summer; imports to this country are supplied mostly by Europe, Mexico, and South America.

Description and assay of *Atropa belladonna* will be found in the *U. S. Pharmacopœia* under *Belladonnae folium* (belladonna leaf) and in the *National Formulary* under *Belladonnae radix* (belladonna root), the method of assay being identical to that of "Hyoscyamus." The method of the *British Pharmacopœia* differs from that of the *U. S. Pharmacopœia* in a number of details. Belladonna leaf is often adulterated with the leaves and tops of *Scopola carniolica* and of *Solanum nigrum*, and with the leaves of Phytolacca and Ailanthus. Adulterants can be detected by microscopic examination of the drug, and methods pertaining thereto have been described by Kraemer [29] and by Guérin and Guillaume.[30] The root is often adulterated with the stem-bases of its own plant or with the roots of Scopola or Phytolacca.[31]

Preparations official in the *U. S. Pharmacopœia* are: Belladonna Leaf, Belladonna Extract, Belladonna Tincture and Belladonna Ointment; but Belladonna Leaf Fluid Extract, Belladonna Root and Belladonna Root Fluid Extract are now found in the *National Formulary*. The *British Pharmacopœia* recognizes Belladonna Leaf, Belladonna Extract, Belladonna Tincture, Belladonna Leaf Fluid Extract, Powdered Belladonna, Belladonna Root, Belladonna Root Fluid Extract, Plaster of Belladonna, and Liniment of Belladonna.

Hyoscyamus is official in both the *U. S.* and the *British Pharmacopœiae*, where their description and methods of assay are described, the British method being quite different from that of the U. S. The *U. S. Pharmacopœia* also recognizes Hyoscyamus Tincture, but Hyoscyamus Extract and Hyoscyamus Fluid Extract are official in both the *National Formulary* and the *British Pharmacopœia*.

Stramonium is also official in the *Pharmacopœiae* of both countries with the description of the leaves in the *British Pharmacopœia* differing from that in the *U. S.* They do not vary materially in their descriptions of the microscopic examination. Differences in the structure of the different species of Datura and their distinction have been described extensively by Timmerman.[32] Adulteration can be determined by microscopic examination, the adulterant being either an excess of stems of Datura or one of the Xanthium species. Besides *Datura stramonium*, Stramonium Extract and Stramonium Tincture are official in the *U. S. Pharmacopœia*, whereas the *British Pharmacopœia* recognizes Stramonium Extract and Stramonium Fluid Extract. The latter is official in the *National Formulary*, which also mentions Stramonium Capsules.

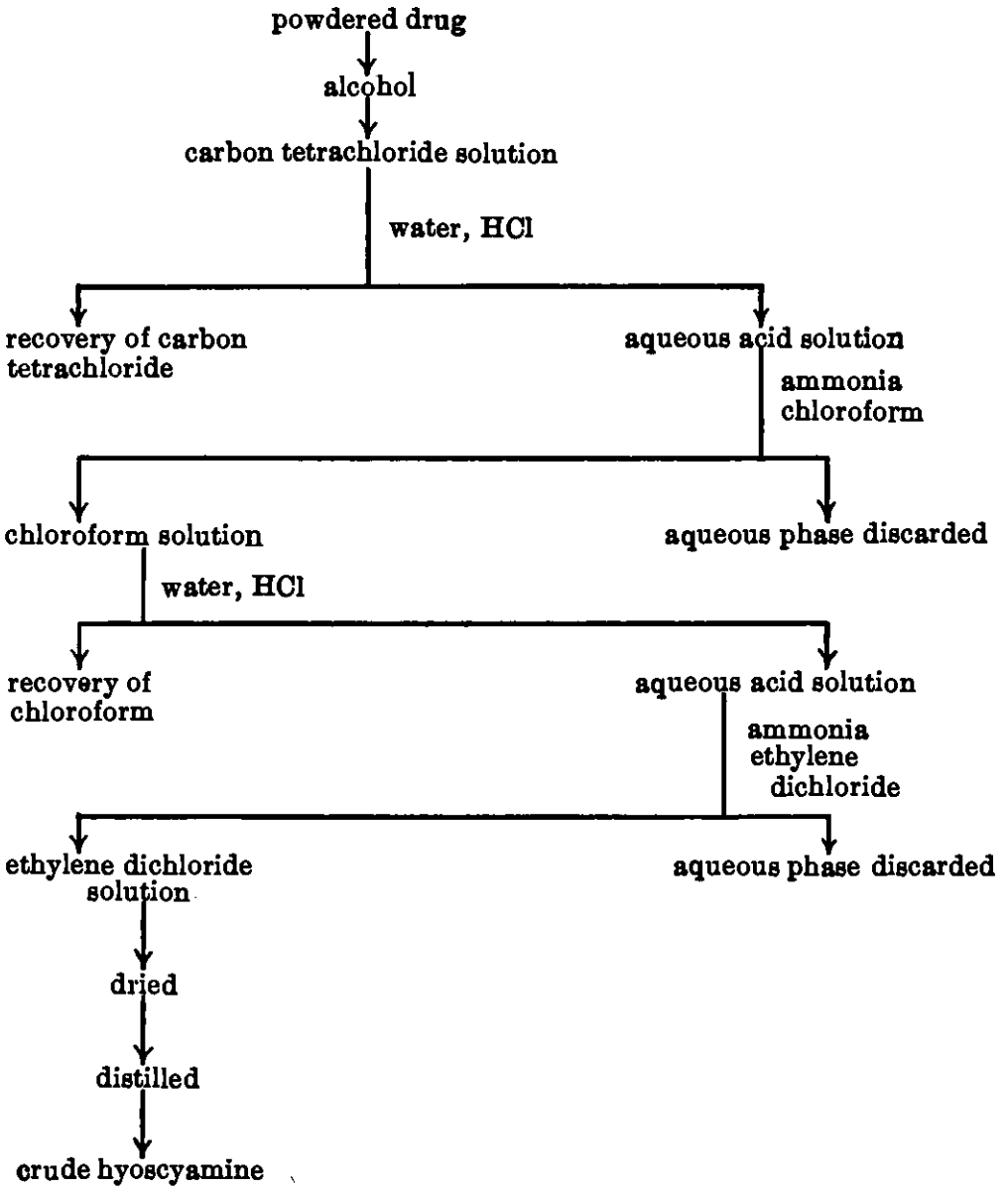
MANUFACTURING METHODS

Hyoscyamine is usually prepared from henbane or other solanaceous plants, whereas atropine in turn is prepared by racemization of hyoscyamine. Atropine probably does not occur in the plant as such, but may be obtained in the process of purification of hyoscyamine by racemization of the latter.[33] This is also borne out by the physiological activity of the powdered drug, which corresponds more closely to that theoretical for hyoscyamine. Scopolamine can be found in the motherliquors from the commercial preparation of hyoscyamine, and the two alkaloids have been separated utilizing preferential extraction with water-immiscible solvents at different pH's,[34] but the usual method of manufacturing is to use *Datura metel* exclusively for the manufacture of scopolamine, that alkaloid being present in the plant almost exclusively.

The isolation of hyoscyamine is usually performed as described roughly

in the diagram (p. 275). Extraction of the ground drug is undertaken without difficulty with alcohol, either hot or cold, as described in Chapter I. The alcohol should preferably be distilled under vacuum, but about two thirds of it could conceivably be removed at ordinary pressure, at which time vacuum should be applied. The solution of the distillation residue in carbon tetrachloride is transferred to a mixing tank, and to it is added about 2–3 times its volume of warm water (60°) and an excess of hydrochloric acid, about 1–1½ lb per each 1000 lb extracted drug. A technical grade of hydrochloric acid can well be used at this point. Stir thoroughly for at least ½ hour and test the supernatant liquor for acidity with congo test paper. This solution should be strongly acid. If not, more acid should be added slowly. Allow to settle for several hours until the carbon tetrachloride has well separated and can be drawn off as the bottom layer. Effect as close a separation as possible and transfer the carbon tetrachloride to mixing tank No. II. Add one half of the previous amount of warm water and a small amount of muriatic acid and proceed to extract as before. If the carbon tetrachloride solution does not separate readily, a small amount of methanol can be added slowly until separation can be effected. After the second extraction the carbon tetrachloride might be tested for alkaloids, but it will invariably be found to be void. It can now be set aside for recovery when convenient. The residue from that recovery can be discarded.

The two aqueous extracts are combined and are allowed to stand and to cool overnight. Decant from any precipitate of tannic acid or filter same with filteraid. Collect the clear liquor and neutralize carefully by the addition of concentrated ammonia until the reaction is slightly alkaline to red litmus paper. The resulting precipitate should be filtered off, as it will interfere with further extraction and reduce the yield, the alkaloids being not very stable in an aqueous solution at that pH. Likewise, the speed of filtration is highly important and it should be undertaken and finished as fast as possible. A filterpress is preferred for that operation, but a large stoneware filter can also be used. To facilitate filtration, the liberal use of filteraid is recommended. The filtercake should be washed well with cold water. The clear, filtered liquor is returned to the empty clean mixing tank, $\frac{1}{10}$ of its volume of ethylene dichloride is added and sufficient ammonia to render it strongly alkaline. Stir thoroughly for ½ hr, allow to settle, draw off the bottom layer of ethylene dichloride, and store on a stoneware crock. Repeat extraction with successively smaller amount of ethylene dichloride, adding small amounts of ammonia each time, until the aqueous phase is free of alkaloids. Four extractions are usually sufficient. Any



emulsion at this point should be separated as fast as convenient by filtering with filteraid.

In this particular phase of extraction chloroform can be used instead of ethylene dichloride. The disadvantages of its higher price are compensated for by its greater solvent power, due to which fewer extractions are necessary. Furthermore, because of its relatively higher specific gravity, separation will be effected speedier and more thoroughly.

The combined solvent extracts are transferred to a mixing tank and are re-extracted with dilute acid, using about ½ of their volume of 5% hydrochloric acid for the first extraction, and a smaller volume and less acid for two more extractions, three being generally sufficient to remove all the alkaloids. The exhausted solvent can be recovered when con-

venient, and the residue can be discarded. If emulsions occur in this series of extractions, they can best be broken by filtration. However, no loss of alkaloid will occur here, because the solanaceous alkaloids are quite stable in an acid solution.

The aqueous acid extracts are combined, made alkaline by the addition of an excess of ammonia, and are extracted with small amounts of ethylene dichloride until exhausted of alkaloids. The solvent extractions are collected and combined in a vessel of suitable capacity, where they are dried with anhydrous sodium sulfate. Filter on a small stoneware filter, wash thoroughly with ethylene dichloride and collect all filtrates in a suitable still.

The combined liquor in the still should be evaporated under reduced pressure. A vacuum of 25 in. or better is desirable for that operation, because the temperature should be kept as low as possible. The hyoscyamine will start to crystallize long before all the ethylene dichloride has been removed. It should not be heated above 45–50°. To remove the last traces of ethylene dichloride, anhydrous methanol can be added, with warming and stirring, until all the hyoscyamine has gone into solution. The rest of the ethylene dichloride can now be removed together with the methanol under reduced pressure at a fairly low temperature. Care should be taken not to contaminate the main amount of recovered ethylene dichloride with methanol. Mixtures of ethylene dichloride and methanol can be partially recovered by washing the methanol out with water and recovering the remaining solvent.

The crystalline residue in the still is crude hyoscyamine. It can be turned into pure hyoscyamine base, hyoscyamine sulfate, or hyoscyamine hydrobromide; or it can be racemized to atropine.

In this last series of extractions isopropyl ether has been used with success instead of ethylene dichloride. Its advantage lies in the fact that it possesses fairly good solvent power with respect to hyoscyamine, but that it will not dissolve many of those impurities which are taken up by ethylene dichloride. A better grade of crude hyoscyamine is obtained in the end, but a greater number of extractions will have been necessary to take up all of the alkaloid present.

The Manufacture of Hyoscyamine Sulfate

The preparation of hyoscyamine sulfate is best undertaken in small quantities in the laboratory.

An appropriate amount of crude hyoscyamine is removed from the still, dissolved in dry methanol at room temperature, and filtered with charcoal, cold. This solution is then cooled to $-5°$ and neutralized with a cold solution of sulfuric acid in methanol as described under

atropine sulfate. The correct amount of sulfuric acid is to be determined by titration with litmus test paper as an indicator. The neutral solution is then treated with charcoal in the cold, filtered, and evaporated under vacuum at as low a temperature as possible. Hyoscyamine sulfate will crystallize from the residue upon addition of acetone and seeding or scratching. If the material separates as an oil, a small amount of methanol should be added to facilitate crystallization. Recrystallization, if necessary, can be effected after treating in cold methanol (10% solution) with charcoal and treating as before.

The Manufacture of Hyoscyamine Alkaloid

An appropriate amount of purified hyoscyamine sulfate (85% alkaloid base, anhydrous) is dissolved in water (10% solution), treated with charcoal cold, filtered, and the hyoscyamine precipitated carefully with a 10% solution of sodium carbonate in water until further addition of sodium carbonate yields no more precipitate. Filter immediately, wash thoroughly with water until the washings are free of sodium sulfate, and dry under vacuum over caustic soda flakes. Screen through a 40-mesh screen after 24 hr and continue drying over fresh caustic soda.

The base, instead of precipitating and filtering, can also be liberated with ammonia, extracted with ethyl or isopropyl ether, and can then be crystallized from those solvents upon concentrating. It must be borne in mind, however, that pure hyoscyamine is not too soluble in those solvents and often commences to crystallize before extraction is finished. To avoid danger of partial racemization in the ethereal solution due to the ammonia present, alkalinization can be undertaken with sodium carbonate instead.

The Manufacture of Hyoscyamine Hydrobromide

Freshly precipitated hyoscyamine alkaloid is dissolved in acetone to form a 10% solution which must be filtered, if cloudy. The alkaloid is then carefully neutralized by the addition of aqueous hydrobromic acid (48%), until neutral to moist litmus paper. Keep in ice chest overnight and allow to crystallize. If the material separates as an oil, addition of a small amount of methanol and scratching will usually bring about crystallization. Filter the hydrobromide and wash with acetone. It is possible to recrystallize this salt from methanol and acetone, but temperatures should be kept as low as possible.

The Manufacture of Atropine Alkaloid

To the crystalline residue of crude hyoscyamine in the still is added enough dry methanol to form a 10% solution. 1–2% of the approximate

weight of hyoscyamine of charcoal is added, and an amount of filteraid, at least equal in volume to that of the charcoal. The solution is brought to boiling and is then filtered, preferably through the pressure filter, the filtercake being washed with hot methanol. This solution of hyoscyamine is now ready for racemization to atropine.

A great deal has been written concerning the mechanism of that reaction. It was found that it takes place with or without heat, and under the influence of alkalies. Will and Bredig [35] ventured the opinion that the racemization of hyoscyamine is a catalytic process, and that any hydrolysis of the alkaloid is a side reaction. They recommend treating the hyoscyamine, dissolved in 66% alcohol, in the cold with a dilute solution of alkali. It was also reported [36] that *l*-hyoscyamine keeps indefinitely in an alcoholic solution, and this could be fully confirmed, although Pictet stated that racemization takes place by letting an alcoholic solution of hyoscyamine stand by itself for some time. It was found that laevo-hyoscyamine dissolved in either pure absolute or pure dilute alcohol, in the cold or by the application of heat, does not racemize. Racemization, however, was effected by the addition of small amounts of alkali. The speed of racemization was found to be in direct proportion to the temperature as well as the specific speed of the catalyst employed, which depended upon its solubility and its ionization. Hydrolysis of the alkaloid took place in direct proportion to the amount of water present in the solvent, as well as temperature and catalyst.

Fairly good yields were obtained by subjecting the crude hyoscyamine to a temperature of from 120–130°, with or without vacuum, for a period of from one quarter to one hour. The atropine was crystallized by adding an equal volume of acetone to the hot syrup, under good stirring and rapid cooling. A second crop was obtained by repeating the foregoing process and adding one half the volume of acetone to the syrup. If the temperature was allowed to rise too high, apo-atropine was formed which was carried through into the final product.

A new method of racemizing hyoscyamine has been devised, with excellent results. The hot solution of hyoscyamine in methanol is transferred to a vessel suitable for refluxing. 1–2% of the weight of hyoscyamine of phenol is added to the solution, the liquor is brought to the point of boiling, and slight refluxing is maintained for 36 hr. At the end of that time a sample is taken to test for optical activity. Enough liquid is taken to contain about 2 grams of alkaloid, and this is brought up to 50 cc with normal hydrochloric acid. Mix thoroughly and filter clear with charcoal. The optical activity should be zero. If this is not the case, refluxing must be continued until all the hyoscyamine

has been converted. Hydrolysis is negligible and racemization is quantitative, but it must be borne in mind that the crude material used here contains on the average only 75% of hyoscyamine.

The hot solution of atropine in methanol is again treated with charcoal and the proper amount of filteraid. The solution is filtered through the pressure filter and the filtercake is washed with hot methanol. The clear filtrates are combined and are evaporated under vacuum (25 in.) until a fairly thick, crystalline syrup is left in the still. To that syrup, while it is still warm, is now added an equal volume of acetone or methyl ethyl ketone, and the mixture is warmed and stirred until all the crystals are loose. The material is taken out of the still and allowed to crystallize, preferably in the icebox, in a suitable container. If the crystalline mass becomes too thick, more acetone or methyl ethyl ketone can be added at will. Filter on a stoneware filter, wash thoroughly with acetone or methyl ethyl ketone to remove all coloring matter, place on trays and dry. The filtrate is processed further as will be described later.

For the manufacture of atropine sulfate this crude atropine alkaloid can be used as is. If chemically pure atropine base is desired, the following recrystallization will often prove sufficient.

An appropriate amount of crude alkaloid, figuring an average yield of 80% of pure material from the crude, is dissolved in dry methanol (about ½ gal per pound) and mixed with charcoal (about 1 oz per pound) and filteraid. The solution is boiled for a very short time and is filtered clean through the pressure filter, washing the filtercake with a small amount of methanol. The clear filtrate and washing are combined, evaporated under vacuum, and the residue crystallized with acetone or methyl ethyl ketone as before.

The filtrates from the preceding recrystallizations, if any, are combined with the previously obtained filtrate and washings from the crude atropine alkaloid. They are placed in a still and evaporated under reduced pressure to a thick syrup. To this syrup is added fresh acetone or methyl ethyl ketone, about twice its volume, and the mixture is warmed until everything is in solution. Place in the icebox and allow to crystallize for at least 48 hr. The atropine base is then filtered off and washed as before. The filtrate and washings are again evaporated under vacuum, but very rarely can an additional crop be obtained. It is more profitable to keep that syrup for the manufacture of homatropine.

The diverse acetone or methyl ethyl ketone distillates obtained contain small amounts of methanol which cannot be separated, but which make the acetone useless for this particular job. The recovered methyl

ethyl ketone, if clean, can be used again until a high concentration of methanol renders it useless also.

The following working times needed for the foregoing operations are approximate: Filtration with charcoal through the pressure filter should take at most one hour. Evaporation of liquors under vacuum should take 1–1½ hr per 10 gal of liquid. Filtration of batches of atropine alkaloid on the stoneware filter should not be hurried. The material should be well pressed down and drained of liquid before and during washing, to save acetone or methyl ethyl ketone. Three to four hours should be allowed for such an operation.

The Manufacture of Atropine Sulfate

The commercial preparation of atropine sulfate from the alkaloid is a rather delicate operation, and the procedure described here should be adhered to closely.

a. Preparation of the Alkaloid. 25 lb of crude atropine alkaloid are dissolved in 12 gal of dry methanol, and are filtered hot with 8 oz of charcoal and filteraid through the pressure filter as described. The filtercake is washed with 2 gal of hot methanol. The combined filtrates are placed in a 25-gal container, either stainless steel or glass-lined, and the container is placed in a wooden tub or other vessel, where it can be thoroughly cooled with ice and salt, or with brine from a small refrigerator. Care must be taken that none of the brine can be splashed into the solution of atropine, which is to be stirred effectively by means of a mechanical stirrer made of stainless steel or glass. Cooling must be so effective that the solution of atropine can be maintained at a temperature not higher than 5° below zero (Centigrade).

b. Preparation of the Solution of Sulfuric Acid. This solution must always be prepared freshly. It can be made 12 hr prior to its use if it can be stored during that period at a very low temperature, near freezing. Two gallons of methanol are cooled to below freezing, and 1050 cc of concentrated sulfuric acid (c.p., sp. gr. 1.84) are added slowly under stirring, maintaining that temperature.

c. Preparation of Atropine Sulfate. This solution of sulfuric acid is now added gradually and cautiously to the cold solution of atropine, maintaining the temperature of the solution at −5° C. This operation will take about 6 hr. When most of the acid has been added, the batch must be tested frequently with moist litmus test paper. The endpoint will be reached when the batch is still slightly alkaline. At that point the red, moist, litmus paper will turn blue, while the blue litmus paper will show a very slight indication of turning pink. A definitely alkaline reaction is always to be preferred to a slightly acid reaction. A slightly

acid batch should not be processed further until that condition has been corrected. When all the acid has been added, stirring should be continued for ½ hr. If at the end of that time the pH is still correct, cooling can be discontinued.

d. Crystallization of Atropine Sulfate. The alcoholic solution of atropine sulfate is treated warm with 8 oz of charcoal and filteraid. The clear filtrates are combined and evaporated to approximately ½ its volume under ordinary pressure and then further on under reduced pressure. When the liquid has thus been concentrated to a heavy syrup of about 5 gal volume, about ⅓ gal methanol is added to it. The mixture is heated and stirred and is immediately transferred to a clean container of at least 25-gal capacity. The still is washed with ⅓ gal of hot methanol, and this washing is added to the batch. Five gal of acetone are now added immediately and are mixed thoroughly with the hot residue without touching the side of the container to prevent crystallization at this time. Another 10–15 gal of acetone are now added as fast as possible, and thorough mixing is continued without scratching the side of the vessel. When the container is practically full, crystallization is started by merely scratching the side once or twice. It will be complete in a few minutes.

After cooling for several hours, the atropine sulfate can be filtered off on a stoneware filter. The filtercake should be pressed down and washed thoroughly with fresh acetone until the washings are colorless. Place on glass trays and dry.

The atropine sulfate thus obtained is usually white, free of optical rotation, and has the correct melting point. If recrystallization appears to be necessary, this can be done from methanol and acetone after treating a more dilute methanolic solution with charcoal. The combined filtrates and washings are evaporated under ordinary pressure. The residue is usually negligible and is used for the manufacture of homatropine. The recovered acetone contains methanol and is useless for further use in the manufacture of atropine sulfate. Methyl ethyl ketone has been substituted for acetone.

Other Salts of Hyoscyamine and Atropine

Other salts of these alkaloids with either organic or inorganic acids can be made easily in alcoholic or acetone solution by mixing equimolecular amounts of alkaloid and acid.

Atropine methylbromide can be made readily in the following manner. Atropine alkaloid is dissolved in a sufficient amount of chloroform or ethylene dichloride, and the mixture is stirred mechanically and cooled with ice. Methyl bromide is then introduced in a slow stream until all

the base has been converted into the methyl bromide. When a sample is filtered, the filtrate should give no precipitation with methylbromide. Atropine methylbromide is allowed to crystallize overnight. It is then filtered, washed with acetone and recrystallized from alcohol and acetone after having treated the alcoholic solution with charcoal and filteraid.

Atropine methyl nitrate is always prepared from atropine methyl bromide. The latter is dissolved in sufficient 90% methanol to make a 15–20% solution. To this solution is then added under mechanical stirring the calculated amount of silver nitrate dissolved in a small amount of distilled water. The precipitated silver bromide is filtered off after about one hour, and is washed on the filter with some methanol. The combined filtrate and washings are evaporated under vacuum at a low temperature to a small volume, and atropine methyl nitrate is crystallized by the addition of acetone. The salt can then be recrystallized from methanol and acetone after treatment with charcoal and filteraid.

The Manufacture of Scopolamine Hydrobromide

Scopolamine occurs, as a rule, in conjunction with hyoscyamine, the latter usually being the predominant alkaloid. In *Datura metel,* however, and in a very limited number of other Datura species, scopolamine is the main alkaloid, and it is that plant which is usually used for its commercial preparation.

Methods for manufacturing scopolamine are essentially alike to those for making hyoscyamine. A number of investigators recommend, however, the use of carbonates or bicarbonates for the liberation of the alkaloid from its salts, possibly to avoid any danger of destroying the oxygen linkage within the scopine molecule or else to minimize racemization to atroscine. Neither side reaction has been observed to take place under the influence of ammonia, provided the concentration of alkaloid was kept below 10%.

Datura metel is extracted with cold or moderately warm methanol or denatured alcohol as described previously. It is recommended, however, to concentrate the combined extracts under vacuum at a low temperature. Dissolving the residue in carbon tetrachloride and extracting same with dilute hydrochloric acid is also undertaken as described under hyoscyamine. The solution is then freed of tannates made alkaline with ammonia and extracted with chloroform. At this stage, a large excess of ammonia is to be avoided. From the chloroform solution the scopolamine is re-extracted into dilute hydrochloric acid. Make alkaline with ammonia (avoid excess), and extract with ethylene dichloride until all alkaloids have been removed. Re-extract with dilute acid, again make alkaline with dilute ammonia, and extract with

isopropyl ether or ethyl ether. Combine the ether extracts, dry thoroughly with anhydrous sodium sulfate, filter, and distil the filtrates at a low temperature. The last traces of ether can be removed by adding a small amount of acetone and removing it under vacuum. The residue is almost pure scopolamine which can be crystallized from dilute alcohol if desired.

For the manufacture of scopolamine hydrobromide, the base is dissolved in three times its volume of acetone, and the solution is thoroughly chilled. Enough of a concentrated aqueous solution of hydrobromic acid (48%) is now added, under constant stirring and thorough cooling, until a slight acid reaction against congo paper is obtained and remains constant. If the mass of crystals becomes too heavy, more acetone can be added. Allow to crystallize in the icebox overnight, filter, and wash well with acetone. Scopolamine hydrobromide can be recrystallized from 70% ethyl alcohol from which it crystallizes with 3 moles of water of crystallization. Filter, wash with 70% alcohol, and allow to dry at room temperature. Both atroscine and scopolamine hydrobromide will be found in the motherliquors. These can be evaporated at a low temperature under vacuum, and another crop of scopolamine hydrobromide can be obtained by renewed crystallization. If only a small amount of atroscine is present, a third crop of scopolamine can often be obtained, which will still show the correct optical rotation. However, if a fairly large amount of atroscine is present, crystallization from 70% alcohol will be unsuccessful, but crystallization of the anhydrous hydrobromides from 95% ethyl alcohol will often show good results, atroscine hydrobromide usually crystallizing out first.

Scopolamine hydrobromide is official in the *U. S. Pharmacopœia*, where its physical characteristics and its assay can be found. It is also found in the *British Pharmacopœia* under *Oculentum hyoscinae*.

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

The pharmacological action of belladonna root and belladonna leaves and their galenical preparations is due chiefly to the presence of hyoscyamine and is very similar to that of atropine. In the human body, atropine has a stimulating effect upon a number of higher nerve centers, but it paralyzes the peripheral endings of certain autonomic nerves (parasympathetic group). It shows a stimulating action on the vasomotor center and, in small amounts, on the medullary center as shown in an increased rate of breathing. Larger amounts act upon the cerebral centers as is evidenced by delirium. Atropine in large doses acts as a depressant, but in smaller doses it can be used to relieve spasmodic

conditions of certain involuntary muscles. To relieve asthmatic paroxysm it can either be administered hypodermically or the fumes of belladonna leaves can be inhaled. Atropine both dilates the pupil of the eye and paralyzes the muscle of accommodation. The autonomic nerves, mentioned previously, carry impulses to organs of unconscious life. They include the oculo-motor nerve, motor nerves to part of the intestines and to the bronchii, and nerves supplying secretory glands, except the kidneys.

The therapeutic effects of Hyoscyamus are very similar to those of belladonna and atropine. It is usually administered, however, in somewhat larger doses. Because of the presence of scopolamine, a central narcotic effect is obtained, but inasmuch as the ratio of the two alkaloids varies in the plant, constant results can only be obtained by administering known mixtures of the two drugs. Hyoscyamus is employed to relieve spasmodic affections of unstriped muscles and to relieve certain forms of nervous irritations, but scopolamine is said to be better for that purpose.

Scopolamine is used mostly as a cerebral sedative in certain maniacal states, delirium tremens and certain types of insomnia, and together with morphine it is used to relieve pain. Scopolamine paralyzes the peripheral endings of the parasympathetic nerves; it dilates the pupil of the eye, causes dryness of the skin, and accelerates the action of the heart. In other words, its action is like that of atropine, but less pronounced. Atroscine is less powerfully still. It shows no effect on the heart, breathing, and secretions, but it will cause dilation of the pupil of the eye. Its action upon the central nervous system is equal to that of scopolamine. Scopolamine has found use in epilepsy, in paralysis agitans, and in postencephalitic Parkinsonism. Together with morphine it is used as an anesthesia to induce twilight sleep.*

Numerous chemical and experimental data indicate that the selective action of atropine resides in the tropic acid part of the ester and depends upon the presence of a free hydroxy group there and esterification with a tertiary amino alcohol. Substitution of other acids for tropic acids results in compounds whose therapeutic action is qualitatively altered. Because tropic acid is an optically active compound, the effects of the dextro- and laevorotatory and of the racemic esters were investigated. Dextro-hyoscyamine was found to possess only weak peripheral actions in the body, and the action of atropine is thought to be due to the presence of laevo-hyoscyamine, the latter having the same action on the central nervous system as atropine but being twice as powerful in its action on the peripheral autonomic nerve endings. Whereas dextro-

* During World War II scopolamine hydrobromide was used extensively to alleviate the symptoms of air- and seasickness.

hyoscyamine is rapidly destroyed in the body, atropine passes through almost unchanged and can often be detected in the urine.

ANALYTICAL AND TOXICOLOGICAL METHODS

Atropine and its salts dissolve without coloration in concentrated sulfuric acid and in aqueous and alcoholic caustic soda. Colorless solutions are also obtained with concentrated nitric acid, Froehde's reagent and with Erdmann's reagent. A dilute solution of vanadium sulfate yields a red color on mixing with a solution of atropine in dilute sulfuric acid. Para-dimethylamino benzaldehyde gives an intensive red color which turns purple on slight warming and standing. Mercuric chloride gives with atropine a yellow precipitate which turns red on heating. With an excess of mercuric chloride, crystals of a double salt with atropine are deposited. Scopolamine can be distinguished because it gives neither a red nor a yellow precipitate with mercuric chloride.[37] Mayer's reagent yields a precipitate in dilute acid solutions, but formation of the precipitate is often hindered by the presence of small amounts of methanol.

Atropine can be detected in microscopic quantities according to Eder[38] or Schoorl.[39] According to the first method, atropine is dissolved in ½ N sulfuric acid, and one drop is placed on a slide. Upon addition of a drop of bromine water microscopic yellow needles are obtained which disappear as the bromine evaporates. The identical result can be obtained by using a solution of potassium bromide, but the latter method is not as sensitive as the first. According to Schoorl, atropine is hydrolyzed on a slide with 30% caustic soda, and the fumes which emanate upon heating are condensed on another slide. To the condensate a drop of dilute hydrochloric acid is added, mixed, and allowed to evaporate. Upon addition of water and a solution of potassium iodide, needles of tropine hydriodide crystallize.

For toxicological investigations, atropine can often be detected in the kidneys or in the urine as well as in the feces. The alkaloid is not decomposed by putrefaction. The tissues are extracted with sulfuric or acetic acid and the alkaloid is precipitated with sodium hydroxide and sodium carbonate and extracted with ether. It can subsequently be titrated with dilute hydrochloric acid, iodoeosine as indicator.[40]

Scopolamine, after evaporation with fuming nitric acid and treatment of the residue with alcoholic potassium hydroxide, gives a yellow to purple color. Atropine, under the same conditions, gives a violet color which changes slowly to dark red and finally disappears.[41] Scopolamine gives a blue color with concentrated sulfuric acid when warm, and, with ammonium heptamolybdate in either dilute hydrochloric or con-

centrated sulfuric acid, it gives a yellow color which changes to blue.

Atropamine can be distinguished from the other solanaceous alkaloids by its sensitivity to potassium permanganate. which it decolorizes.

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CHAPTER XIX

VERATRINE

CHEMISTRY

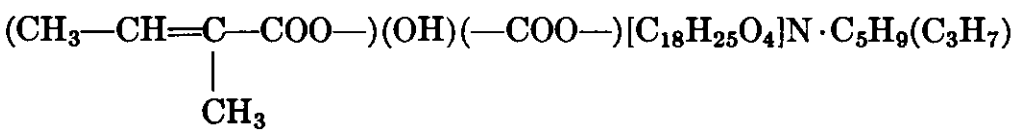
Commercial veratrine is a mixture of several alkaloids of sabadilla seeds, consisting mainly of cevadine and veratridine. The former is also known as "crystalline veratrine." Sabadine and cevadilline are minor alkaloids found in commercial or "amorphous" veratrine, as well as sabadine. The term "amorphous veratrine" has also been applied to veratridine itself. Cevadine and veratridine are also found in the green hellebore, *Veratrum viride.*

Cevadine, $C_{32}H_{49}O_9N$, has been isolated by Ahrens,[1] Schmidt and Koppen,[2] Wright and Luff,[3] and G. Merck.[4] It crystallizes from alcohol with two moles of that solvent of crystallization, which is lost by drying to 140°.[5] Its melting point is 205° (anhydrous); its optical rotation varies according to the solvent used (alcohol: +12.5°; pyridine: +6.4°; acetone: +1.25°).[6] It is insoluble in water, sparingly soluble in petroleum ether and ether, soluble in warm alcohol, and in ethyl acetate, chloroform, acetone and amyl alcohol. Most of its salts are crystalline: aurichloride (m.p.: 190°); picrate (m.p.: 225°); mercurichloride (m.p.: 172°).

The alkaloid was found to contain no methylimino or methoxyl groups, to yield a benzoyl derivative (m.p.: 255°) and a ortho-nitrobenzoyl derivative (m.p.: 236°), also a methiodide (m.p.: 212°), which can be converted into methylcevadinium hydroxide.[7] With alcoholic hydrochloric acid an ester exchange takes place with the formation of cevine and ethyl tiglate.[8] Cevadine is hydrolyzed under the influence of alcoholic alkali into cevine, angelic and tiglic acids.[5]

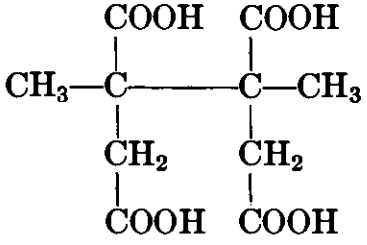
The empirical formula of cevine has been established as being $C_{27}H_{43}O_8N$,[9] and has been obtained in crystalline form by Freund and Schwarz.[5] The compound has m.p.: 198–200°, α_D −17.5°. It forms potassium derivatives with alcoholic potassium hydroxide and it reduces Fehling's solution. It gives rise to a dibenzoyl derivative (m.p.: 196°), a diacetyl derivative (m.p.: 190°), and a di-ortho-nitrobenzoyl derivative (m.p.: 175°). It forms a hydrochloride (m.p.: 247°), an aurichloride

(m.p.: 162°), and a methiodide (m.p.: 257°), from which a phenolic or enolic betaine can be prepared.[10] Cevine is said to contain a methylene dioxy group,[11] and to form an oxide under the influence of hydrogen peroxide.[12] Distillation with soda lime gives rise to *l*-coniine,[13] and the following partial formula for cevine has been suggested by Macbeth and Robinson:[13]



According to Jacobs and Craig,[10] distillation of cevine with soda lime gives rise to the following products: dextro-beta-pipecoline, with some of the laevo compound also present; 5-methyl-2-ethyl-piperidine; and a tertiary, bicyclic base, $C_{10}H_{19}N$ (picrate, m.p.: 150°), which is thought to be a methyl-octahydro-pyridocoline or a dimethyl-octahydro-pyrrocoline. The following products of the distillation with zinc-dust have been identified: dextro-N-methyl-beta-pipecoline and beta-pipecoline; a base thought to be 5-methyl-2-ethyl-pyridine, and a base, $C_9H_{13}N$ (picrate, m.p.: 142°), not identified.[14]

Craig and Jacobs have also investigated the oxidation of cevine with chromic acid and have found the following acidic saponification products:[15] the dimethyl ester of methyl-succinic acid and the methyl ester of succinic acid; an acid, $C_{11}H_{14}O_8$ or $C_{11}H_{16}O_8$, which could be easily saponified, contained two methyl ester groups, two carboxylic acid and one lactone group; a tetra methyl ester, $C_{14}H_{12}O_8$ (m.p.: 66°), which could be hydrolyzed to a hexanetetracarboxylic acid, $C_{10}H_{14}O_8$ (m.p.: 175°), which in turn formed an optically active dianhydride and at high temperature and pressure gave a keto-mono-anhydride. Since the acid was found to contain two tertiary carboxyl groups, its structure was suggested to be as follows:



Also present among the products of oxidation was 4-methyl-2-pyrrolidone and 5-methyl-2-piperidone and 2-ethyl-5-methyl-piperidine or pyridine.

The same authors also found a compound, $C_{14}H_{14}O_6$ (m.p.: 278°), which could be esterified with diazomethane forming two methoxyl

groups, $C_{16}H_{18}O_6$ (m.p.: 166°). The compound is thought to contain two labile lactone groups. It gives a positive reaction with ferric chloride and adsorbs three moles of hydrogen upon hydrogenation. It probably contains a benzol ring and is possibly a lactone of a substituted tetrahydronaphthalene.

Dehydrogenation of cevine with selenium also gives rise to a number of products:[16] beta-picoline, 2-ethyl-5-methyl-pyridine, 2-hydroxyethyl-5-methyl-pyridine, another oxygen containing ethylmethyl pyridine, possibly a cyclic ether, 4,5-benzahydrindene, and cevanthrol and cevanthridine. The last two compounds have been described previously by Blount.[17] The former is possibly a 3-hydroxy-substituted-phenanthrene.[18] It behaves as a phenol and gives an acetyl derivative. Cevanthridine may be a phenanthrene derivative with a fourth benzene ring fused on.

Veratridine, $C_{36}H_{51}O_{11}N$ (m.p.: 160–180°, α_D +7.2°), has been described by Schmidt and Koppen,[19] Wright and Luff,[3] Merck,[20] Bosetti,[21] and Blount.[17] It can be separated from cevadine by chromatographic adsorption on aluminum oxide,[22] when the latter can be eluted with benzol and the former with chloroform. Hydrolysis with alcoholic potassium hydroxide yields cevine and veratric acid.[3,17]

Cevadilline, also known as sabadilline ($C_{34}H_{53}O_8N$)[3] and sabadine ($C_{29}H_{51}O_8N$)[23] occur in very small amounts only.

As mentioned previously, cevadine and veratridine are also found in *Veratrum viride* Ait, the green hellebore, where it is found in the rhizomes. These alkaloids do not occur in the white hellebore, *Veratrum album* Linné, but because of the latter's close relationship to the former, the alkaloids of the white hellebore shall be mentioned briefly. Very little is known of the constitution of the Veratrum alkaloids, but they are thought to be closely related to the Solanum alkaloids, and to be alkaloidal steroid or glycosides.[24] Much contradictory information concerning the Veratrum alkaloids has been published.[25]

The formula for jervine has recently been modified by Jacobs and Craig to $C_{27}H_{39}O_3N$.[26] It has m.p.: 245°, α_D −150°, is soluble in alcohol acetone and chloroform, sparingly in ether. Its salts are crystalline. According to Saito *et al.*,[27] the base contains two active hydrogens, yields a tetrahydro derivative, a diacetyl compound, a N-methyl derivative and a nitroso derivative, and it is thought to contain a methylene dioxy group. Its dehydrogenation products have been studied by Jacobs *et al.*,[28] who also described its isolation.[29]

Pseudojervine, $C_{33}H_{49}O_8N$, has been isolated from the plant after removal of the other alkaloids by extraction with ammonia and benzol.[30] The base has m.p.: 307°, whereas the hydrochloride has m.p.: 256°.

The alkaloid is a secondary base, contains four hydroxyl groups, five active hydrogen atoms, no methoxyl and no methylenedioxy group. The alkaloid is thought to be the *d*-glucose glycoside of jervine. Hydrolysis of the alkaloid yields isojervine, isomerization probably taking place during the hydrolysis.

In the motherliquors of the foregoing alkaloid, a new base was found by Jacobs and Craig,[30] $C_{33}H_{49}O_7N$ (α_D $-53°$), which could be hydrolyzed to *d*-glucose and a base, $C_{27}H_{39}O_2N$ (α_D $-69°$).

Rubijervine and isorubijervine, $C_{27}H_{43}O_2N$, have also been isolated and described by a large number of authors.[31] They are tertiary bases, probably containing two hydroxyl but no methoxyl or methylenedioxy groups. Both alkaloids give the Rosenheim color reaction for allocholesterol and probably have a double bond in the four-position. Through the corresponding ketones they can be transformed into the alio- and epiallo-compounds. Dehydrogenation of isorubijervine gives 1,2-cyclopentenophenanthrene, indicating a normal sterol ring system without nitrogen.

Other alkaloids isolated from *Veratrum viride* are veratramine, $C_{27}H_{39}O_2N$, germine, $C_{27}H_{43}O_8N$, and a new alkaloid, $C_{27}H_{41\text{ or }39}O_4N$.[32] Veratramine forms a triacetyl derivative which can be saponified to N-acetyl-veratramine. The new alkaloid is a polyhydroxy secondary base, which forms an N-acetyl and a nitroso derivative, but no oxime.

Germine is obtained by alkaline hydrolysis of germerine, $C_{36}H_{57}O_{11}N$, and of protoveratridine, $C_{31}H_{49}O_9N$, both of which are found in the white hellebore. Other products obtained by the hydrolysis of germerine are laevo-ethylmethyl-acetic acid and methylethyl-glycollic acid. Cautious hydrolysis of germerine yields protoveratridine and methylethyl-glycollic acid. Protoveratrine, $C_{40}H_{63}O_{14}N$, found in the same plant, can be hydrolyzed to acetic acid, methylethyl-acetic acid, methylethyl-glycollic acid, and protoverine, $C_{27}H_{43}O_9N$. For details of the chemistry of all these alkaloids, the reader is referred to the original literature.[33]

NATURAL SOURCES

The main source of veratrine is the seeds of *Asagroea officinalis* (Cham. and Schl.) Lindley, identical with *Schoenocaulon officinale* (Schlecht.). Of secondary importance is *Veratrum viride* Aiton. The dried seeds are used from the former, but the dried rhizome is used from the latter. The seeds are also known as sabadilla or cevadilla seeds.

The plant *Schoenocaulon officinale* is a native of the mountainous districts of Mexico, Guatemala, and Venezuela. It grows wild, but it is also cultivated. The fruit of this bulbous herb is a three-celled cap-

sule, and in each locule are found from one to six albuminous seeds, three or four on the average. The elongated seeds are pointed at the ends, somewhat flattened on one side, and convex on the other. They are 5–8 mm long, somewhat curved, and often have a beak-like formation on one end. They are wrinkled, glossy, brownish-black on the outside, whitish and hard on the inside, and have a small linear embryo at the rounded end opposite the beak. When powdered, the drug is a powerful sternutatory.

Veratrum viride Aiton, green or American hellebore, is official in the *National Formulary* (8th Edition). To distinguish this plant from *Veratrum album* Linné is rather difficult.[34] The parts used are the dried rhizome and roots of the plant, which is 3–6 ft high, and annual. The leaves are bright green and decrease in size near the top. The cylindrical stem is topped by a panicle of yellow-greenish flowers. The plant is usually found in damp places, swamps, meadows, and near streams. The rhizome is erect, thick, and fleshy and has numerous whitish roots. It should be dried very carefully, because it deteriorates very easily.[35] Commercial supplies are obtained from Michigan, Illinois, Virginia, and North Carolina.

MANUFACTURING METHODS

The sabadilla seeds, prior to extraction, must be powdered or crushed. Crushing of the seeds in a roller mill is usually sufficient and is preferable to powdering because of the physical and physiological disadvantages of the latter operation. It is important that the hull of the seeds be broken and the inside exposed. Although a major part of the alkaloids probably occurs in the epidermis of the seeds, it is important that the inside be permeated by the solvent. The powdered drug, besides being an extremely strong sternutatory, is also poisonous, and it is important that the personnel engaged in this operation be well protected with dustproof masks, protecting the eyes as well as the nose. Crushing of the seeds will generate less dust, and, because it is sufficient, it is to be preferred.

The crushed seeds are then charged into a percolator and are extracted with methanol or ethanol, which has been acidified with 1% tartaric acid. Extraction should be undertaken warm, and circulation of the liquid is indicated. Four extractions are usually sufficient to remove all the alkaloids. Then if a sample of the drug after drying and extraction with dilute hydrochloric acid yields no precipitate with Mayer's reagent, it can be discarded.

The alcoholic extracts are combined and evaporated to a syrup, first under ordinary pressure and finally under vacuum. The syrupy residue

remaining in the still is dissolved in about five times its volume of carbon tetrachloride or ethylene dichloride, and this solvent solution of the distillation residue is transferred to an extractor.

The alkaloids are now removed from this solution by repeated extraction with dilute sulfuric acid (1%), until a sample of the aqueous acid solution gives no more precipitate with sodium carbonate solution. Depending on how effective a separation of aqueous and solvent phase can be achieved, three to four extractions should be sufficient. Emulsions can be broken by slight warming, very gentle stirring, the addition of very small amounts of methanol, or filtration with filteraid. The filtercake must be washed thoroughly with solvent as well as with dilute sulfuric acid. These aqueous acid extractions are combined, and, if so desired, the last traces of fat or resin can be removed by extraction with petroleum ether.

The combined aqueous acid extracts are made alkaline with either sodium carbonate, potassium carbonate, or ammonia, and the total alkaloids which precipitate are extracted with chloroform. With good stirring and time allowed for settling, three to four extractions are usually sufficient. For the first extraction, about one fifth of the aqueous volume of chloroform is needed; for the successive extractions, succeedingly smaller portions of chloroform are used. Ethylene dichloride has been used instead of chloroform, but one or two additional extractions are often necessary, and there seems to be a greater tendency toward the formation of emulsions.

The total solvent extractions are carefully dried with anhydrous sodium sulfate, filtered, and the filtercake washed with chloroform. The combined filtrates and washings are distilled under vacuum to a small volume, about one-half to three-quarters gallon per 100 lb of crude drug. This concentrate is now introduced in a thin stream into an excess of petroleum ether, under constant and rapid stirring. At least ten times the amount of the chloroform concentrate by volume is needed for that operation. The precipitate is filtered off, washed with pentane, and dried.

To effect purification of this crude mixture, it is dissolved in dilute acetic acid (10%), treated with a small amount of charcoal, which should be free from metals, and with filteraid. After filtration and washing of the filtercake with water, the alkaloids are precipitated with ammonia in the presence of a trace of sodium hydrosulfite. Any unnecessary excess of ammonia should be avoided. This process of purification can be repeated until the total alkaloids are nearly colorless. The use of dilute ammonia is important for the precipitation of the alkaloids, and the fact that an excess must not be used to avoid serious discolorations.

A modification of this procedure of purification is to dissolve the precipitated alkaloids in ether and to allow them to crystallize from that solvent. Because of the rather low solubility of the alkaloids in ether, a considerable amount of that solvent is necessary. After drying and concentrating the solution, the alkaloids are permitted either to precipitate from the ether or precipitation is induced and hastened by the addition of small amounts of petroleum ether. Under these conditions, a separation of cevadine and veratridine can often be effected.

Veratrine was official in the *National Formulary* (5th Edition), where it was described as follows: "Veratrine is a white or grayish-white, amorphous powder, odorless but causing intense irritation and sneezing when even a minute quantity reaches the nasal mucous membrane. Great caution must be used in handling it. It is slightly hygroscopic. One gram of veratrine dissolves in 1760 cc of water, 2.8 cc of alcohol, 0.7 cc of chloroform, and 4.2 cc of ether at 25° C: also in 1345 cc of water at 80° C; insoluble in petroleum benzine."

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

The action of cevadine is somewhat similar to that of aconitine. It stimulates the ends of the sensory nerves, giving rise to a prickling sensation of the skin. On the mucous membranes, violent attacks of sneezing are followed by a feeling of numbness. Internally, it intensifies and prolongs the contractions of the striated muscles. Taken internally, it causes vomiting and great general discomfort. Similar to aconitine, the central nervous system is first excited and then depressed; death may be caused by respiratory failure. The heart rate is also slowed by stimulation of the cardio-inhibitory center. Removal of the acyl group from cevadine results in a considerable lowering of the toxicity. Veratridine and the commercial veratrine resemble cevadine in their action. The main use of veratrine in medicine is as an anodyne counterirritant in various forms of neuralgia and arthritis, but care must be taken to avoid adsorption of too much of the alkaloids through the skin. An oleate and an ointment of veratrine were recognized by the *National Formulary* (5th Edition).

The alkaloids of white and green hellebores differ from that of veratrine. They have found some use as insecticides, but the original literature should be consulted for details.

ANALYTICAL METHODS

Cevadine has been variously reported either to give no coloration with concentrated hydrochloric acid or to give a purplish violet which

turns red on heating. Concentrated sulfuric acid gives a yellow color, which turns red on heating and shows a green fluorescence. Mixed with sugar and sulfuric acid, a green color is obtained which changes to blue. Veratridine remains colorless in hydrochloric acid; it gives a red color with blue fluorescence in sulfuric acid.

With sulfuric acid, commercial veratrine yields a yellow color which changes to purple; with concentrated nitric acid, a yellow color; with Erdmann's and Froehde's reagents and with benzaldehyde and sulfuric acid, a yellow color changing to red. Hydrogen peroxide and sulfuric acid give an orange color; formaldehyde and sulfuric acid give a yellow to red brown color; fuming hydrochloric acid gives a deep red color. Precipitates are obtained with potassium iodide, Mayer's reagent, phosphomolybdic acid and tannic acid (1:5000), aurichloride and picric acids (1:1000), potassium dichromate and platinic chloride (1:500).

A number of methods, both chemical and biological, for the quantitative estimation of the alkaloids, have been published.[36]

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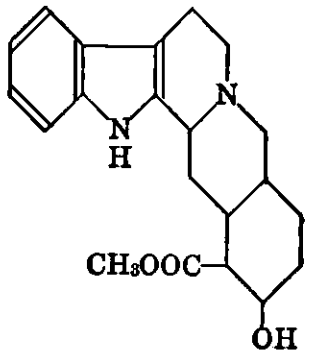
CHAPTER XX

YOHIMBINE

CHEMISTRY

Yohimbine is the main alkaloid of yohimbé bark, and of its several isomers it is the only one of commercial importance. Its identity with quebrachine is now generally accepted, and this is of great interest in view of the fact that the latter base occurs in a species of plants belonging to a different order than those in which yohimbine is found.

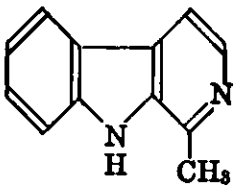


Yohimbine

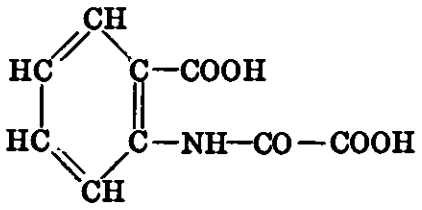
The foregoing formula of yohimbine has been confirmed recently, but little is known of the constitution of the secondary bases of yohimbé bark, most of which are probably optical isomers of yohimbine. At least one of them, corynantheine, has been found to accompany yohimbine as well as corynanthine, one of the yohimbine isomers occurring in a different species.

In the course of investigating the structure of yohimbine, Barger and Field [1] and Warnat [2] obtained a base, $C_{10}H_{12}N$ (m.p.: 56°), the former from yohimbine and the latter from allo-yohimbine, by distillation with soda lime, and their findings were confirmed by Winterstein and Walter.[3] This base was thought to be either an ethyl or a dimethyl-indole, but comparison with isomers synthesized by Warnat [2] and by Mendlik and Wibaut [4] has not been successful. Permanganate oxidation of yohimbine yields a N-oxalylanthranilic acid, identified by Spaeth and Bretschneider,[5] and *o*-hydroxy phenylcarbimide (m.p.: 200°), identified by Warnat.[6] Both these compounds indicate the presence of an indole

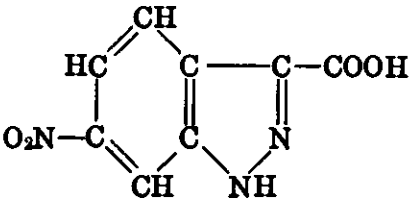
nucleus, and further proof was obtained by Hahn and Just [7] who obtained 6-nitroindazole-3-carboxylic acid from di-O,N-acetylyohimbine. From yohimbic acid, of which yohimbine is the methyl ester,[8] Warnat [9] and Winterstein and Walter [10] obtained a base which was subsequently identified as harman (m.p.: 232°), $C_{12}H_{10}N_2$ by Barger and Scholz.[11]



Harman



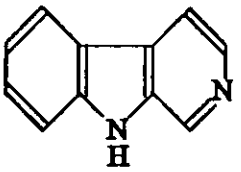
N-oxalylanthranilio acid



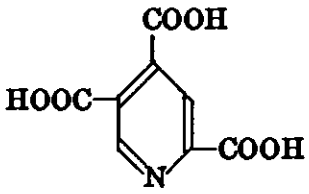
6-Nitroindazole-3-carboxylic acid

It was indicated above that yohimbine is the methyl ester of yohimbic acid. This monocarboxylic acid is isomeric with those obtained from yohimbene, meso-(or iso-)-yohimbine and gamma-yohimbine, but all four acids ($C_{20}H_{24}O_3N$) [12] yield the same carbinol, yohimbol, $C_{19}H_{24}ON_2$ (m.p.: 307°) upon dicarboxylation.[13] These four acids probably differ in their stereochemical configuration. In a similar series of reactions allo-yohimbine yields allo-yohimbol, but alpha-yohimbine yields neither allo-yohimbol nor yohimbol. Another proof of the close relationship of the aforementioned four alkaloids becomes apparent from the fact that identical substances are obtained by dehydrogenation with selenium.[14] They are yobyrine, $C_{19}H_{16}N_2$ (m.p.: 218°), tetrahydroyobyrine, $C_{19}H_{20}N_2$ (m.p.: 167°), and ketoyobyrine, $C_{20}H_{16}ON_2$ (m.p.: 330°). Tetrahydroyobyrine was also obtained by the alkaline fusion of yohimbic acid together with *m*-toluic acid, 3-ethylindole, and indole-2-carboxylic acid. Upon oxidation, tetrahydroyobyrine yields berberonic acid, while yohimbic acid under similar conditions yields succinic acid, and yobyrine yields *o*-phthalic acid. On the other hand, ketoyobyrine on alkaline hydrolysis yields nor-harman and 2,3-dimethylbenzoic acid, which ascertains the position of the carboxy group.

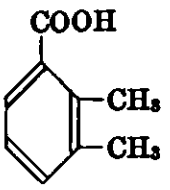
In addition to the reactions described, Winterstein and Walter [15] observed the formation of tetrahydroisoquinoline from tetrahydroyobyrine, and Mendlik and Wibaut [16] observed dimethylbenzoic acid



nor Harman



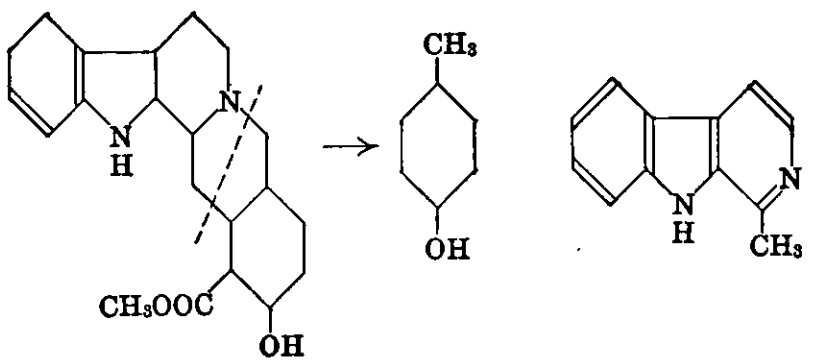
Berberonic acid



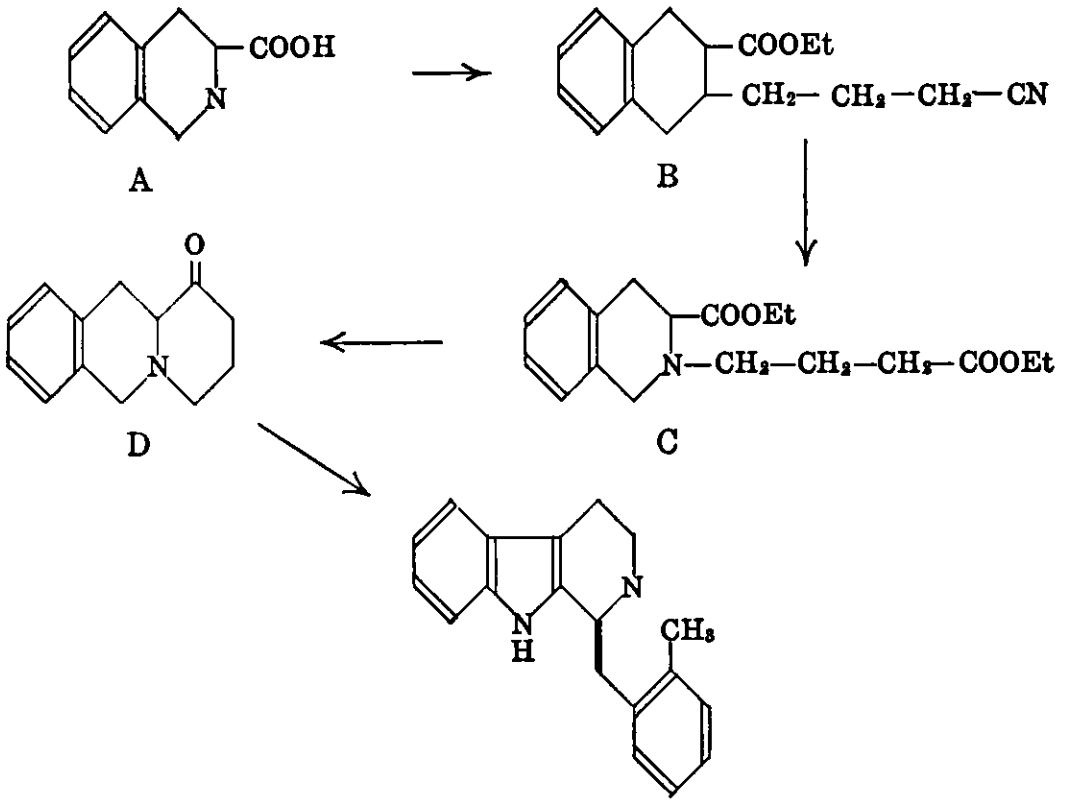
2,3-Dimethyl-benzoic acid

as one of its fusion products. Other oxidation products of tetrahydroyobyrine are *o*-aminopropiophenone, berberonic acid, and 5,6,7,8-tetrahydroisoquinoline-3-carboxylic acid.[17] Yobyrine, on oxidation, yields phthalic acid, *o*-toluic acid and a keto-compound, which can be transformed into an amphoteric acid. Based on the reactions mentioned previously, formulae for yohimbine and for yobyrine were suggested by Barger and Scholz,[18] by Scholz,[19] by Wibaut and Gastel,[20] and by Hahn and Werner,[21] but these have been somewhat revised to conform with some recent observations.

Witkop [22] found that yohimbol reacts with thallium hydroxide at 300°, forming the ketone yohimbone and desoxyyohimbol, $C_{19}H_{24}N_2$. Yohimbone was also obtained in 90% yield by the Oppenauer oxidation of yohimbine with sodium phenolate and cyclohexanone without the formation of apoyohimbol, thus suggesting the presence of a beta-hydroxy-acid. Since the position of the carboxyl group had previously been fixed at C-16, the hydroxyl group had to be located at C-17. Proof for this assumption was given by the zinc-dust distillation of yohimbine, which gave para-cresole and harman.



Witkop (*loc. cit.*) also suggested a formula for yobyrine, which was confirmed by Clemo and Swan [23] by a total synthesis of that compound. Phenylalanine with formaldehyde gave 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (A),[24] which was condensed with gamma-propylcyanide, forming ethyl-2-(gamma-cyanopropyl)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (B). Diekman reaction of the corresponding carbethoxy compound (C) gave 1-keto-7,8-benzo-1,2,3,4,6,9-hexahydropyridocoline (D), whose phenylhydrazone through the Fisher indole reaction gave yobyrine, 7,8-benzo-1,2-(2′3′-indolo)-3,4,6,9-tetrahydropyridocoline.



Yobyrine

Previous confirmation of the position of the carbomethoxy group at C-16 had been given by Hahn, Kappes, and Ludewig,[25] who investigated the action of lead tetra-acetate on yohimbine. and by Majima and Murahasi.[26]

NATURAL SOURCES

Yohimbine was first thought to be derived from species of Tabernoemontana (Natural Order of Apocynaceae), but it is now known to be produced from *Corynanthe yohimbe* Schm., also known as *Pausinystalia yohimbe* (K. Sch.) Pierre, and from *Pausinystalia trillesii* (Natural Order of Rubiaceae).[27] Quebrachine, which is identical with yohim-

bine,[28] is derived from *Aspidosperma quebracho* Schlecht, which belongs to the family of Apocynaceae. Corynanthine, together with corynantheine, is found in *Pseudocinchona africana* Chev., also known as *Corynanthe africana* (Br.) [28] (or possibly *Corynanthe paniculata*).[29] Of all these plants, *Corynanthe yohimbe* and related species are the only ones of commercial importance.

This rubiaceous tree is indigenous to the Cameroon district of western Africa, but it is also found in the French Congo. The only part of the plant used is the bark, which occurs in commerce, in strips of about 2 ft long and about 2–3 in. wide. The individual pieces may be flattened out, or slightly bent and quilled. They are about 4–10 mm thick, and vary in color from red to grayish-brown or brown. The external corky layer is often covered with isolated lichens.

The outer surface shows longitudinal furrows in addition to smaller transverse cracks which appear at regular intervals of somewhat less than one inch. Old bark is usually thicker than young bark, and often darker in color. The transverse fissures are absent or not very numerous as compared with a younger bark. Fractures show a soft velvety surface.

The drug of commerce is often adulterated with bark from *Pausynistalia macroceras* Pierre (*Corynanthe macroceras* K. Sch. Brandt.), which contains mostly physiologically inactive yohimbinine;[30] with *Pausynistalia paniculata* Welw., which contains some yohimbine together with paniculatine,[31] and with *Pausinystalia trillesii* Pierre.[32]

"Under the microscope transverse sections of the outer zone of the bast shows a beaded appearance, but the bark of *Pausynistalia macroceras* (K. Sch.) Pierre, offered as yohimbé bark, differs in arrangement of bast fibers which in the outer zone are in tangential groups of two." [33]

The powdered bark of *Corynanthe yohimbe*, when shaken with a very dilute solution of sodium hydroxide, gives a red color at once. Substitutes usually give a yellow color which becomes red on standing.

MANUFACTURING METHODS

For the manufacture of yohimbine the bark is powdered and extracted with alcohol.

Powdering of yohimbé bark is somewhat more difficult than powdering of most drugs because of the toughness and fibrous condition of the material, but the obstacle is not an insurmountable one. A cutting mill of the type of feed mills described in a different chapter gives excellent service as a rule, although the knives and the screen will need more care than usual. If possible it will be found advantageous to have two sets of knives, so that one set can be sharpened while the other set is in

use. Knives as well as the screen will need replacement relatively more often than when the mill is used for other drugs.

The powdered drug is rather voluminous and of a velvety or fibrous appearance, but it extracts well with alcohol, and the loss of solvent is kept at a minimum because of excellent drainage.

Either methanol, ethanol, or isopropanol can be used for extraction with equally good results. Acidification with organic acids has been recommended, but it was found not to be necessary. The extractions are undertaken warm, and, as a rule, three or four extractions are sufficient to remove all the alkaloids. Because of the condition of the material, because of the necessity for relatively larger amounts of solvent, and because of the easy extractability of the alkaloids, the system of countercurrent extraction yields excellent results.

After the fourth extraction the drug can be tested for alkaloids by extracting a dried sample with dilute hydrochloric acid, and adding to the clear filtrate a few drops of Mayer's reagent or of phosphomolybdic acid. Absence of a precipitate, even if a cloudiness is present, indicates that the drug is void of alkaloids and can be discarded.

The alcoholic extracts are combined in the still and are evaporated. Two thirds of the solvent can be removed at ordinary pressure, but, near the end, vacuum should be applied with continuous stirring to avoid overheating.

The syrupy residue is dissolved in carbon tetrachloride or in ethylene dichloride. This operation will need prolonged stirring and some heating. The resulting solution is then blown over into the mixing tank as described previously.

The total alkaloids are now removed from that solution by several extractions with dilute hydrochloric or sulfuric acid (5%). Three extractions are usually sufficient. Emulsions occurring at this stage can be broken either by filtration or by addition of small amounts of methanol. The aqueous acid extractions are combined and are allowed to stand overnight, after which time, as a rule, tannins and other foreign acid-insoluble material separate. These materials can be removed by filtration. Because of the slimy condition of this precipitate, the addition of filteraid and, for large quantities, use of a filterpress are indicated. The clear filtrate and washings are combined, and concentrated ammonia is added under constant stirring until the solution is slightly alkaline to litmus test paper. At this point a copious precipitate, consisting of inert material together with a small amount of yohimbine, is formed. This is removed by filtration after having been mixed with a large amount of filteraid. A large stoneware filter is to be preferred for this operation. The filtercake is drained as much as possible. It is then

transferred to a mixing tank where it is mixed with three times its volume of chloroform or ethylene dichloride, and the suspension is stirred thoroughly for one hour. The solvent is then filtered off, and the extraction is repeated once more, after which operation the filtercake should be free of yohimbine. The clear, solvent filtrates are put aside. The exhausted filtercake can be discarded. The filtercake is tested for alkaloids by dissolving a sample of the same in dilute hydrochloric acid, filtering, mixing the filtrate with ether and making it alkaline with ammonia under continuous shaking or stirring. The ether is then separated and tested for the presence of alkaloids.

The slightly alkaline aqueous filtrate obtained is now made strongly alkaline with ammonia, and the total alkaloids are removed by repeated extraction with chloroform or ethylene dichloride. The latter is preferred because of its selective solubility action on the bases. Usually three extractions, or four at the most, are sufficient to remove all the bases. Occasionally emulsions are formed at this stage also. They are broken by filtration, and the filtercake is extracted with solvent as described previously. All the solvent extractions are collected and are combined with those obtained from the filtercake mentioned previously. They are dried thoroughly with anhydrous sodium sulfate or with anhydrous potassium carbonate, filtered, and distilled under reduced pressure to a thick syrup.

This syrup consists of a mixture of yohimbé alkaloids, but yohimbine predominates. It can be prepared in a pure state, if so desired, by crystallization from benzole and final recrystallization from dilute alcohol. The usual article of commerce, however, is the hydrochloride, which is prepared in a pure state directly from the crude mixture of bases. This can be accomplished because of the greater solubility of the hydrochlorides of the secondary bases in acetone or other ketonic solvents, such as methylisobutyl ketone.*

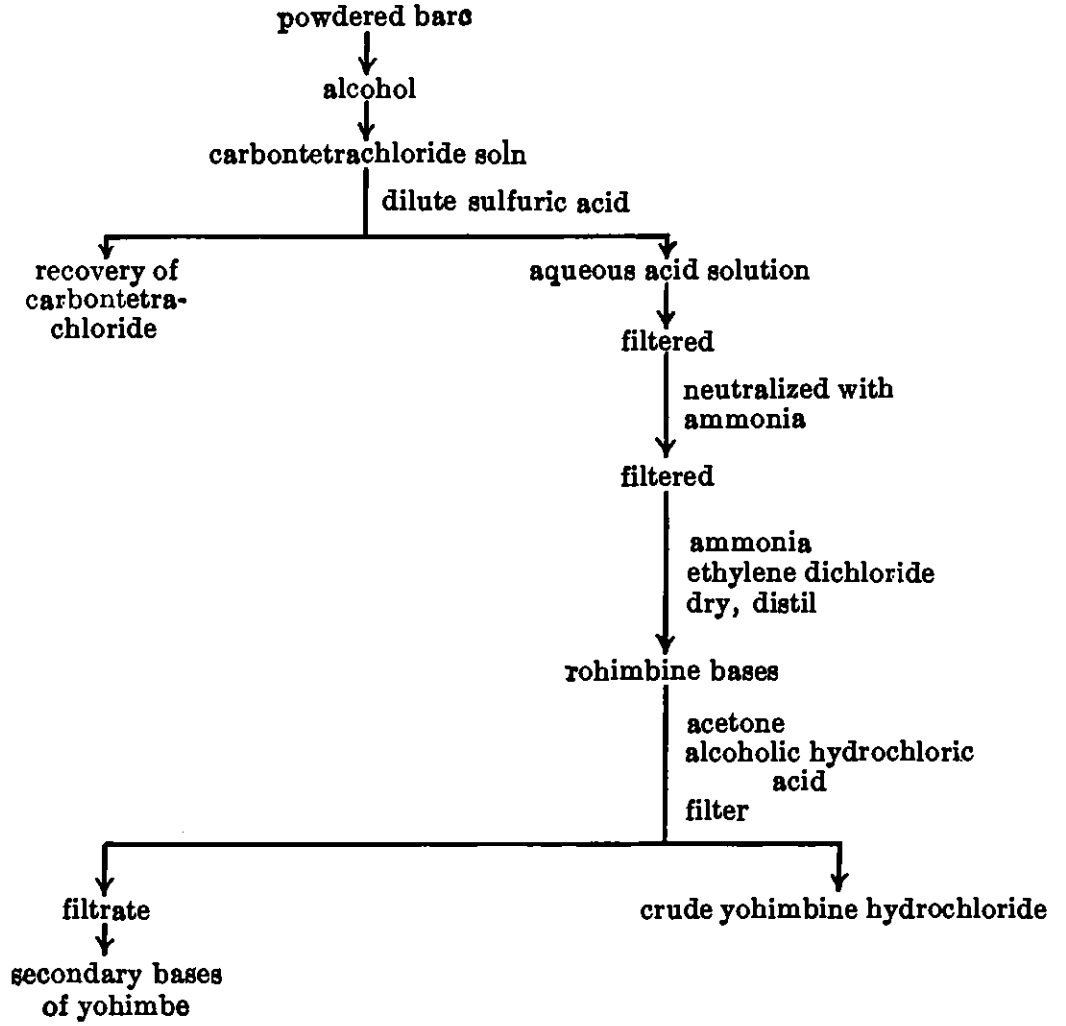
The crude yohimbé alkaloids are dissolved in ten times their volume of acetone or methyl ethyl ketone, and the solution is transferred to a glass-lined container equipped with a stirrer and cooled thoroughly with ice or brine. A strong solution of alcoholic hydrochloric acid is slowly added with continuous stirring until the liquid shows a very slight but definite reaction to congo test paper. An excess of alcoholic hydrochloric acid is to be avoided, to forestall a possible loss of yohimbine hydrochloride. The latter starts to crystallize almost immediately upon addition of the acid, and, as the suspension of the material becomes thicker, more acetone or methyl ethyl ketone should be added as necessary. After addition of the acid is complete, stirring is continued for

* Private communication from Mr. R. L. Brown.

several hours, and the material is then allowed to crystallize in the cold overnight. It is then filtered and washed thoroughly with acetone or methyl ethyl ketone. The material thus obtained after drying is almost white or white, but often shows some discoloration after keeping for several weeks.

Yohimbine hydrochloride can be recrystallized from hot, dilute alcohol after treating with charcoal in the pressure filter. The material which crystallizes from this solution is pure and white and shows no tendency toward discoloration. A second crop of yohimbine hydrochloride can be obtained from the motherliquors by dilution with 10% aqueous hydrochloric acid.

Occasionally yohimbine lactate can be found in commerce. This salt is best prepared from pure yohimbine hydrochloride. A solution of that salt in dry methanol is mixed with a solution of sodium lactate in the same solvent. The precipitate of sodium chloride is filtered off, and the clear motherliquor is evaporated to dryness. Yohimbine lac-



tate can be recrystallized from methanol on the addition of acetone and ether.

PHARMACOLOGICAL PROPERTIES AND THERAPEUTIC USES

Yohimbine is the most active alkaloid of those of the yohimbé bark.[34] Second in potency is aspidospermine (from *Aspidosperma quebracho*). Most of the other bases are much less potent or show no action whatsoever.

Yohimbine stimulates the respiratory center, in respect to both amplitude and frequency, leading to paralysis in excessive doses. If death occurs from that cause, the heart will continue to function for some time. Yohimbine also has some influence on the blood pressure, probably by causing the blood vessels to dilate, thus lowering the blood pressure.[35] It thus acts as an antagonist to epinephrine [36] and has been shown to be more effective than ergotoxine [37] in that respect. To a limited extent it also has some local anesthetic properties.

The alkaloid is said to be a strong aphrodisiac, and Mueller [38] ascribes its action to an increased excitability of the lower centers of the spinal cord in addition to the hyperemia produced in the genital regions. The main use of yohimbine at this time is as an aphrodisiac in veterinary medicine.[39]

A considerable amount of information has also been collected on the physiological properties of the secondary alkaloids of yohimbé, not only in comparing their aphrodisiac action with that of yohimbine, but also in investigating on a comparative basis their anesthetic properties and even their vasodilating action.[40]

ANALYTICAL METHODS

Yohimbine can be determined quantitatively by both gravimetric and volumetric methods.[41] The following method developed by Vogt and King [42] gives good results.

50 grams of powdered yohimbé bark are mixed thoroughly with 30 cc of ammonia (sp. gr. 0.91), pressed through a screen, and are then refluxed for one hour with 270 cc of chloroform. The mixture is filtered and extraction is repeated with 220 and 200 cc of chloroform. The combined extracts are concentrated to 100 cc, and the residue is extracted four times with 30 cc 1% acetic acid each time. Some sodium sulfite is added to the aqueous acid solution. The solution is filtered and washed once with 50 cc ether. The solution is then made alkaline with ammonia and extracted exhaustively with ether. The combined ether extracts are

washed with water, dried with anhydrous sodium sulfate and distilled. To the residue is added 3 cc of absolute alcohol, and four drops of concentrated hydrochloric acid, and the yohimbine hydrochloride is allowed to crystallize. It is filtered, washed with 5 cc of a mixture of alcohol and ether 1:3, dried, and its weight determined. Its melting point should be no lower than 290–295°.

Yohimbine gives a dark blue precipitate with Erdmann's reagent, which on standing turns green and then yellow. It gives a colorless solution with concentrated nitric and sulfuric acid. The former turns yellow on standing; the latter develops blue streaks which turn green upon the addition of a crystal of potassium dichromate.[43] Vanadium salts yield a dark blue color, which later turns green and then red. Ammonium molybdate gives a dark blue color, whereas phosphomolybdic acid yields a yellowish-white precipitate. Millon's reagent yields a deep brown-red color. Picric acid gives a yellow precipitate.

Martini has described the microchemical identification of yohimbine,[44] and he found the Stephenson and Parker test with potassium cyanide to be most sensitive. Microcrystalline precipitates were also obtained by him with sodium borate, sodium selenite, sodium tellurite, and potassium oxalate. In a reverse of the procedure, yohimbine could also be used for identifying the foregoing substances.

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